A DISSERTATION ON

COMPARISON OF SINGLE SPUTUM SMEAR (HOME COLLECTION) BY FLUORESCENT MICROSCOPY VS TWO SMEARS BY ZIEHL NEELSEN METHOD (ROUTINE RNTCP)

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CERTIFICATE

This is to certify that this dissertation entitled **"COMPARISON OF SINGLE SPUTUM SMEAR (HOME COLLECTION) BY FLUORESCENT MICROSCOPY VS TWO SMEARS BY ZIEHL NEELSEN METHOD (ROUTINE RNTCP)"** submitted to the Tamil Nadu Dr.MGR Medical University is in partial fulfillment for the requirement of the award of the degree M.D. GENERAL MEDICINE (BRANCH I) is the original and bonafide work done by **Dr.S.GEETHALAKSHMI** under the guidance and direct supervision of Dr. **S.MAGESHKUMAR**,M.D., Professor, Department of MEDICINE at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of her course in M.D from May-2010 to April-2013 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai - 600032.

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I solemnly declare that this dissertation titled, **"COMPARISON OF SINGLE SPUTUM SMEAR (HOME COLLECTION) BY FLUORESCENT MICROSCOPY VS TWO SMEARS BY ZIEHL-NEELSEN METHOD (ROUTINE RNTCP)"** is the original and bonafide work done by me under the guidance of **PROF.** Dr. **S.MAGESHKUMAR**, M.D., Professor, Department of MEDICINE, at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of my course in M.D. from May-2010 to April-2013 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai - 600032.

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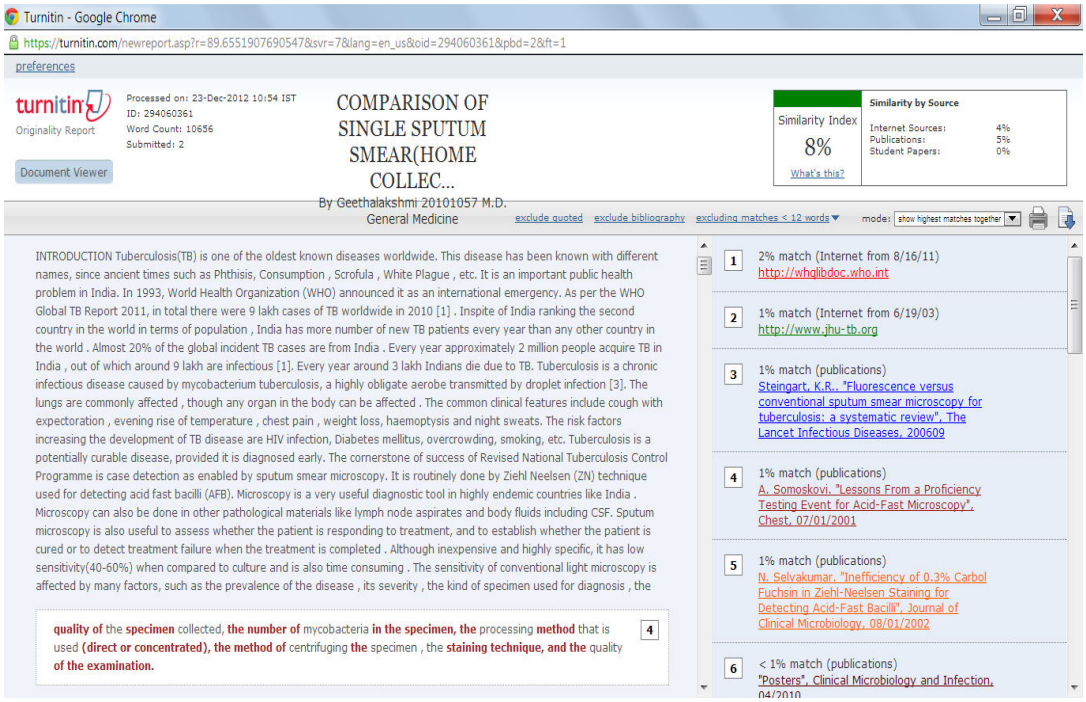
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INTRODUCTION Tuberculosis(TB) is one of the oldest known diseases worldwide. This disease has been known with different names, since ancient times such as Phthisis, Consumption , Scrofula , White Plague , etc. It is an important public health problem in India. In 1993, World Health Organization (WHO) announced it as an international emergency. As per the WHO Global TB Report 2011, in total there were 9 lakh cases of TB worldwide in 2010 [1] . Inspite of India ranking the second country in the world in terms of population , India has more number of new TB patients every year than any other country in the world . Almost 20% of the global incident TB cases are from India . Every year approximately 2 million people acquire TB in India , out of which around 9 lakh are infectious [1]. Every year around 3 lakh Indians die due to TB. Tuberculosis is a chronic infectious disease caused by mycobacterium tuberculosis, a highly obligate aerobe transmitted by droplet infection [3]. The lungs are commonly affected , though any organ in the body can be affected . The common clinical features include cough with expectoration , evening rise of temperature , chest pain , weight loss, haemoptysis and night sweats. The risk factors increasing the development of TB disease are HIV infection, Diabetes mellitus, overcrowding, smoking, etc. Tuberculosis is a potentially curable disease, provided it is diagnosed early. The cornerstone of success of Revised National Tuberculosis Control Programme is case detection as enabled by sputum smear microscopy. It is routinely done by Ziehl Neelsen (ZN) technique used for detecting acid fast bacilli (AFB). Microscopy is a very useful diagnostic tool in highly endemic countries like India . Microscopy can also be done in other pathological materials like lymph node aspirates and body fluids including CSF. Sputum microscopy is also useful to assess whether the patient is responding to treatment, and to establish whether the patient is cured or to detect treatment failure when the treatment is completed . Although inexpensive and highly specific, it has low sensitivity(40-60%) when compared to culture and is also time consuming . The sensitivity of conventional light microscopy is affected by many factors, such as the prevalence of the disease , its severity , the kind of specimen used for diagnosis , the
quality of the specimen collected, the number of mycobacteria in the specimen, the processing method that is used (direct or concentrated), the method of centrifuging the specimen , the staining technique, and the quality of the examination.
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CONTENTS

INTRODUCTION

Tuberculosis(TB) is one of the oldest known diseases worldwide. This disease has been known with different names, since ancient times such as **Phthisis, Consumption , Scrofula , White Plague**, etc. It is an important public health problem in India. In 1993, World Health Organization (WHO) announced it as an international emergency. As per the WHO Global TB Report 2011, in total there were 9 lakh cases of TB worldwide in 2010 [1] . Inspite of India ranking the second country in the world in terms of population , India has more number of new TB patients every year than any other country in the world . Almost 20% of the global incident TB cases are from India . Every year approximately 2 million people acquire TB in India , out of which around 9 lakh are infectious [1]. Every year around 3 lakh Indians die due to TB.

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Tuberculosis is a potentially curable disease, provided it is diagnosed early. The cornerstone of success of **Revised National Tuberculosis Control Programme** is **case detection** as enabled by sputum smear microscopy. It is routinely done by Ziehl Neelsen (ZN) technique used for detecting acid fast bacilli (AFB).

Microscopy is a very useful diagnostic tool in highly endemic countries like India . Microscopy can also be done in other pathological materials like lymph node aspirates and body fluids including Cerebro Spinal Fluid. Sputum microscopy is also useful to assess whether the patient is responding to treatment, and to establish whether the patient is cured or to detect treatment failure when the treatment is completed . Although inexpensive and highly specific, it has low sensitivity(40-60%) when compared to culture and is also time consuming[19]. The sensitivity of conventional light microscopy is affected by many factors, such as the prevalence of the disease, its severity, the kind of specimen used for diagnosis, the quality of the specimen collected, the number of mycobacteria in the specimen, the processing method that is used (direct or concentrated), the method of centrifuging the specimen , the staining technique, and the quality of the examination. So , when microscopy is done accurately, a lot of time is consumed . The Ziehl Neelsen (ZN) method

used at present is a significant modification of Robert Koch's original method using alkaline methylene blue .

Fluorescent microscopy has been used for detection of acid-fast bacilli since Auramine O based method was introduced by Hagemann and Richard et al [4] . It is easy to identify fluorescent bacilli against a dark background . Thus using the fluorescent microscopy (auramine / rhodamine), the examiner can go through the slide at a lesser magnification and observe a large area than that is seen with Ziehl Neelsen -stained smears. The above factors decrease the time required for screening a slide and lead to an increase in sensitivity. Thus , it is widely accepted that the fluorochrome method must be preferred over the Ziehl Neelsen (ZN) method.

There are several reports indicating that fluorescent microscopy (either auramine phenol or auramine rhodamine) of smears significantly increases the sensitivity of direct microscopy. Although many researchers have investigated the significant difference in sensitivity between ZN and FM staining methods, the technical and procedural factors can influence the sensitivity of each staining method.

At present, in India under Revised National Tuberculosis Control programme (RNTCP), the use of fluorescence microscopy is linked to the

culture and Drug Sensitivity Testing (DST) activities at the level of the Intermediate Reference Laboratories (IRL).

In this study, the sensitivity, specificity, efficacy and other advantages of the conventional gold standard ZN method and Flourescent microscopy are compared with each other in the detection of acid fast bacilli in sputum and the results analysed.

AIMS AND OBJECTIVES

1. To assess the value of fluorescence microscopy in diagnosing sputum smear positivity among chest symptomatics when compared to conventional light microscopy by Ziehl Neelsen method for diagnosing cases of pulmonary tuberculosis.
2. To assess the value of fluorescent microscopy in picking up additional smear positive cases among smear negative chest symptomatics.
3. Whether the objectives one and two can be achieved with the single home collection sample compared with routine two smear (Ziehl Neelsen) RNTCP smear microscopy.
4. Age and sex distribution of patients with smear positive pulmonary tuberculosis
5. Additional parameters are also compared such as the incidence of smear positive pulmonary tuberculosis in
   a. Diabetics
   b. Smokers

REVIEW OF LITERATURE

Tuberculosis is a disease that has ravaged mankind for centuries. Although pulmonary tuberculosis was endemic in the middle ages, it transformed into a serious public health problem since the era of Industrial revolution. Overcrowded housing , poor working conditions, alcoholism and malnutrition have contributed to its rapid spread throughout the world. Hence tuberculosis is not only a disease, but is also a marker of economic health of a society. The case fatality rate of tuberculosis has always been around 50% in most of the endemic countries [3].

In many countries there was a steady decline in mortality from tuberculosis, even in the sub Saharan Africa till the early 1990s. This natural decline was the result of many co - ordinated efforts, due to improvement in socio - economic conditions, mass BCG vaccination and the efficacy of anti tuberculous drugs. But this trend has reversed in the recent past and the incidence is again on the rise due to HIV pandemic. On the other hand , it has given rise to the problem of multi drug resistant tuberculosis and also to the emergence of diseases caused by atypical mycobacteria.

RNTCP

In India , in the past - the burden of tuberculosis was very heavy and could not be controlled by multitude of measures taken by the government.

Finally, since 1993 the Government of India implemented the WHO recommended DOTS (Directly Observed Treatment Short course) strategy by means of the **Revised National Tuberculosis Control Programme (RNTCP)** [2]**.** The objectives of RNTCP are: (a) To achieve 85% cure rate among New Smear Positive pulmonary TB cases , (b) To achieve at least 70% case detection rate . RNTCP is under the direct control of Ministry of Health and Family Welfare , Government of India. These goals are achieved by following measures. RNTCP has established approximately around 12,000 microscopy centres across India . DOTS centers are established wherein health workers directly administer the anti tuberculosis drugs to the patient . This ensures that the patient is under constant follow up and the treatment is not interrupted during the 6 month or 8 month period . The programme contains a complete and comprehensive standardised protocol for the training of staffs and guidelines regarding technical and treatment aspects . Operational research is also encouraged at various levels. This has resulted in significant increase in case detection and cure rates . So far about 10 million TB patients have been successfully treated under RNTCP . The case fatality rates have also come down from 29 % to 5 % . In addition to this , the Government has implemented the WHO recommended **Stop TB Strategy** since 2006 which is concerned with the newer issues and challenges that are to be faced in the control of tuberculosis .

RNTCP has also successfully initiated DOTS PLUS programme for the treatment of multi drug resistant Tuberculosis . RNTCP is also aided at the international level by World Health Organization (WHO) both financially and technically by WHO consultants at the field level .

HISTORY OF TUBERCULOSIS

Tuberculosis has been described in many ways in ancient literature. But its nature as an infectious disease was established when Robert Koch identified the elusive microbe. Robert Koch, discovered mycobacterium tuberculosis in 1882 and described its morphology , as deduced from a complex procedure of staining [5]. Shortly thereafter , Rudolph Virchow, the father of cellular pathology, described the development of caseation in tuberculous tissue. In 1890, Koch developed tuberculin and described Koch's phenomenon. Calmette and Guerin produced attenuated Bovine mycobacteria (BCG) in 1921 after sub culturing for about two hundred and thirty times over a period of thirteen years from 1908 - 1921.

Pulmonary TB is the most common manifestation of tuberculosis caused by Mycobacterium tuberculosis. The major route of infection is inhalation of the infectious sputum droplets suspended in air . Generally a single bout of cough from an infectious patient can produce about 3000

droplet nuclei which can stay viable in air for a very long time. In persons with extrapulmonary tuberculosis and sputum negative pulmonary TB , the risk of transmitting the infection is very low. The concentration of droplet nuclei present in the contaminated air and inhalation of the contaminated air for a prolonged period are some of the factors that increase the risk of exposure and acquisition of infection. Other mycobacteria such as Mycobacterium africanum and M.bovis can also cause pulmonary tuberculosis rarely in immunocompromised individuals.

BACTERIOLOGY [3]

Mycobacteria (fungus like bacteria) are slender rods that sometimes form a mold like pellicle in liquid cultures and hence the name. They are aerobic organisms , non motile and non sporing. They are generally slow growing. When cultivated in vitro, they take two to eight weeks to form colonies. Electron microscopic pictures show a thick three layered cell wall rich in mycolic acid enclosing a semi permeable cell membrane.

PATHOGENESIS OF PULMONARY TB

The most frequent portal of entry for mycobacterium tuberculosis is Lung. The organism enters the lung through inhalation of the infectious droplet nuclei. The bacteria reach the alveoli , phagocytosed by tissue macrophages and the bacilli multiply intracellularly. In about four – six

weeks , cell mediated immunity and delayed hypersensitivity develop. As a result , a tubercle is formed containing an area of central caseous necrosis surrounded by granulation composed of giant epitheloid cells , macrophages, lymphocytes and other cell types. This primary lesion, called as **‘ GHON’S FOCUS ’** is usually sub pleural in location. **GHON’S COMPLEX ’ or ‘ PRIMARY COMPLEX ’**.

CLINICAL FEATURES

The various manifestations can be classified as constitutional , pulmonary and extra pulmonary . The constitutional symptoms include low grade fever often presenting during late afternoon or evening , loss of weight , loss of appetite , night sweats and malaise . The most common respiratory symptom is productive cough lasting for more than two weeks followed by haemoptysis , chest pain and breathlessness . Extra pulmonary manifestations include disseminated tuberculosis, tuberculous lymphadenitis , cold abscess , POTT’S SPINE , TB meningitis , abdominal and urogenital tuberculosis .

COMPLICATIONS OF PRIMARY TB

In almost all the affected individuals , the primary complex gets spontaneously resolved without manifesting clinically . This is usually the course in patients with a good immune status. In few patients , primary

pulmonary tuberculosis can progress immediately if the patient is immunocompromised. In others , the primary lesion can remain dormant for years together and get re activated when there is some breach to the immune system of the host. Complications that can arise from the primary focus include **cavitation**, dissemination of the bacilli via lymph and haematogenous route causing **miliary TB ,** involvement of bronchial mucosa leading on to **endobronchial tuberculosis** or cause involvement of pleura producing **pleural effusion or empyems thoracis**

COMPLICATIONS OF PULMONARY TB

In general , the complications of pulmonary tuberculosis are classified as **LOCAL and SYSTEMIC** .

The local complications include,

a. Haemoptysis ,
b. Post TB bronchiectasis ,
c. Endobronchial tuberculosis,
d. Intra cavitary fungal ball (Aspergilloma) ,
e. Spontaneous pneumothorax ,
f. Secondary pyogenic infections ,
g. Disseminated calcification,
h. Fibrothorax,

i. Scar carcinoma , and

j. Broncho pleural fistula .

The **systemic** complications include

a. Secondary Amyloidosis ,

b. Respiratory failure and

c. cor pulmonale.

DIAGNOSIS OF PULMONARY TB

The absolute diagnosis and confirmation of pulmonary tuberculosis requires the detection and isolation of Mycobacterium tuberculosis from the infected host .

Haematolgical abnormalities that can be identified through a routine Complete Blood Count include anaemia , lymphocytosis and elevated ESR.

RADIOLOGY :

In patients with symptoms and signs suggestive of Pulmonary Tuberculosis , a standard Chest X ray in Postero - anterior and Lateral views should be obtained . The usual radiological manifestations include parenchymal infiltrates with ipsilateral hilar lymph node enlargement . Cavitation often occurs in immune competent individuals . Other radiographic findings in pulmonary tuberculosis include consolidation ,

atelectasis , fibrosis , pleural effusion , pleural thickening , pleuro parenchymal calcifications , bronchiectasis,etc.

Patients with Endo bronchial tuberculosis usually have a normal Chest X ray , but such lesions can be identified on CT scan of the chest as a characteristic " **TREE IN BUD** " pattern . It also helps to identify occult or subtle parenchymal disease (eg : - miliary TB) . It also helps to evaluate mediastinal and hilar lymphadenopathy and to identify pathology of central air ways such as bronchiectasis , bronchial stenoses and broncholiths .

LABORATORY DIAGNOSIS

In India , according to Revised National Tuberculosis Control Programme (RNTCP) , all patients presenting with cough and sputum for more than three weeks must be referred for sputum examination by microscopy for mycobacterium tuberculosis (Acid Fast Bacilli) . For patients with scanty sputum production , other respiratory specimens such as induced sputum , laryngeal swab , bronchial washings , trans tracheal aspirate and gastric aspirates can be used.

Sputum microscopy is the earliest and quickest procedure for diagnosing pulmonary Tuberculosis. It also has epidemiological importance in regard to contagiousness of the patient to others . Usually an early morning sputum specimen has a higher yield of AFB. Instead a 12 hour or

24 hour sputum sample can be collected and be centrifuged and concentrated by various methods. Sputum should be collected in a well ventilated room in a wide mouthed container with tight fitting top. If the patient is unable to produce sputum , its production can be induced by inhalation of aerosol of warm hypertonic saline. **Patrick Murray et al**, conducted a study in which he concluded that microscopy for detecting AFB is a highly specific test for tuberculosis but it must be done under proper supervision and also by assuring quality control so that the number of false positives are reduced [6].

CULTURE OF MYCOBACTERIA

The yield of mycobacteria from culture is high. It is highly sensitive and specific and the **GOLD STANDARD** technique . But owing to the long time taken for mycobacterial growth (4- 6 weeks) , it is not the first preferred method for diagnosis. The routine solid medium used for mycobacterial culture is egg based Lowenstein Jensen medium as recommended by International Union Against Tuberculosis (IUAT). Other routinely used culture media include ATS medium and Middle Brook medium . The growth of Mycobacterium tuberculosis can be morphologically confirmed by egg white or buff coloured minute colonies and biochemically by a positive reaction for production of niacin , nitrate , pyrazinamidase and a neagative reaction for catalase .

Another broth – based system is the recently used **SEPTI – CHEK AFB** which combines broth and solid media into a single device.

A novel system for detection of growth is **Mycobacterial Growth Indicator Tube** (MGIT). It contains enriched Middle Brook medium along with an oxygen sensitive fluorescent sensor which indicates mycobacterial growth. It also helps to identify drug resistant mycobacteria and also helps in indirect drug susceptibility studies.

Another procedure unique to the detection of mycobacterium tuberculosis is **BACTEC** liquid culture system. The underlying principle is that p-nitro a -acetylamino b –hydroxypropiophenone used will selectively inhibit the growth of mycobacterium tuberculosis. Here the growth index can also be recorded by measurement of radio activity of carbon di oxide produced from metabolism of radio active C_{14} - palmitic acid used as substrate. It is a rapid test which provides the result in less than five days.

Other rapid identification tests useful in the detection of Mycobacterial tuberculosis include : **High Performance Liquid Chromatography , Gas Liquid Chromatography , mycobacteriophage typing** , **use of genetic probes , Amplified Mycobacterium Tuberculosis Direct Test , sequencing of 165 r RNA of mycobacterial genome ,**

Polymerase Chain Reaction – Restriction Fragment Length Polymorphism and Immuno assay of mycobacterial antigens. Most of these techniques are still in their infancy.

HISTORY OF SPUTUM MICROSCOPY[7]

During the same time period as Koch's discovery of tubercle bacillus by a complex staining technique around 1880 s , many other researchers (Ehrlich, Ziehl, Neelsen and Rindfleisch) suggested modifications to the reagents and the procedure thus improving on the Koch's method.

Franz Ziehl , a German scientist was the first person who used carbolic acid (i.e. phenol) as the mordant. Another scientist **Friedrich Neelsen ,** retained Ziehl's mordant, but he changed the primary stain to basic carbol fuchsin which was first utilized in 1882 by Ehrlich). This method later came to be called as **the Ziehl - Neelsen method** in the 1890s. In this method, the primary carbol fuchsin stain moves into the waxy impermeable cell wall of the mycobacteria due to heat . Therefore this technique is also known as the " **hot staining** " method.

The Ziehl - Neelsen method has evolved into an effective way to detect the acid-fast bacilli especially in low income countries with a high burden of tuberculosis , wherein molecular techniques are still in the developing phase . In 1915, Kinyoun published a method called as the "

cold staining ” method. In this method, the heating step was removed instead of which a greater concentration of the carbol fuchsin primary stain was used.

PRINCIPLE BEHIND THE DETECTION OF ACID FAST BACILLI

There are three staining methods commonly used to diagnose acid fast bacilli namely the Ziehl-Neelsen (hot staining) , Kinyoun (cold staining) , and Auramine - Rhodamine or Fluorochrome (Truant method) methods. Acid fast staining of mycobacteria was first discovered by Erlich in 1882 and later modified by Ziehl Neelsen. The primary stain is retained by Mycobacteria even after exposing them to the decolorising agent , acid – alcohol. Hence the term ' acid-fast ' is used. This is because of the tight binding of the primary stain and phenol to the waxy mycolic acid in the cell wall of mycobacteria. The stained organisms are highlighted by using a counter-stain for easier recognition. But there must be a minimum of 5000-10,000 bacilli in stained smears for identification of acid fast bacilli.

There are many methods to determine the acid - fast nature of mycobacteria. In the routinely employed Ziehl-Neelsen procedure, the acid-fast organisms are identified as red rods on a blue background, whereas in the fluorochrome procedures (auramine-O or a combination of

auramine and rhodamine), the acid-fast mycobacteria appear as fluorescent bacilli , greenish yellow or orange (the colour differs according to the filter system used) on a darker background.

Smears that are stained by Fluorescent dyes can be **restained** by Ziehl - Neelsen method to confirm our previous observations. To restain the same smear for Ziehl - Neelsen , 5% oxalic acid is added to the slide for 2 min, washed and then Ziehl – Neelsen can be done . But , once if smears have been stained by Ziehl - Neelsen method , Fluorescent Microscopy cannot be used to re examine the slides .

COMPARISON AMONG THESE PROCEDURES

Somoskovi, et. al.[8] evaluated the routine performance of the various acid fast staining techniques across 167 laboratories with New york state permits. A comparison among the various techniques using standard statistical analysis revealed a higher sensitivity for flurochrome staining. Moreover, Ziehl Neelsen and Kinyoun's method had no major differences in sensitivity. But only Kinyoun method reported false-negative findings in AFB - positive slides. As a result , there is a significant chance that potentially infectious patients will be reported as smear negative while using Kinyoun's method especially those with a lower grade of smear positivity. It was concluded that the Ziehl Neelsen and fluorochrome methods are

superior to Kinyoun method , even if it is performed according to the recommendations.

Myrna T. Mendoza et. al.,[9] found that the use of fluorescent staining with sputum microscopy gives greater accuracy. Totally 2182 smears were examined , out of which 159 smears were positive for acid-fast bacilli by Fluorescent microscopy and 132 were positive by Ziehl Neelsen microscopy. By both these methods , the total positive yield from the specimens was 159 or 7.0%. Both of these techniques agreed that 2024 (92.7%) smears were negative . The total yield from the 2182 specimens was slightly greater by fluorescent microscopy i.e. 159 or 7.0% when compared to 132 or 6.0% positivity by the Ziehl neelsen method. Higher grades of smear positivity were noted when using Fluorescent microscopy . Fluorescent microscopy picked up 24 more positive AFB smears (1.09%) which were completely missed by the Ziehl neelsen method. Higher yield of positivity by Fluorescent microscopy was noted which suggest that this technique has greater sensitivity . High grade of positivity was also observed using fluorescent microscopy , which is very similar to the H.L. David study that was cited by Toman. The sensitivity was 50% and specificity was 97% using Ziehl neelsen method when compared to that of fluorescent microscopy (sensitivity 65.4% and specificity 96.2%). Due to the lower magnification (250 X, 400 X) used to scan fluorochrome stained

smears when compared to Ziehl neelsen method (1000 x), the technician could scan a much larger area of the sputum smear in a shorter span of time. In addition to saving time, fluorescing yellow orange bacillus is easier to detect a dark background . These results revealed that it is a very effective technique for detection of AFB in centers with a huge work load for sputum microscopy. They also suggested that fluorescent microscopy should be the preferred choice for sputum AFB detection in mass diagnostic surveys.

F. Ba, H. L. Rieder et. al.,[10] compared fluorescent and conventional microscopy for detection of tubercle bacilli. In the period between January 1996 and June 1998, two smears were prepared from 2630 consecutive sputum specimens. Out of the two smears, one was examined by the Ziehl-Neelsen technique and other by fluorescent microscopy at 1000x magnification. The time needed to screen a slide and report it as negative was calculated for both techniques for 68 slides. Concordance was 96.9% and 92.3% for diagnostic and follow-up examinations, respectively. The results were identical for both techniques for samples with a minimum of 10 bacilli per 100 fields, but higher with fluorescent microscopy in those slides with lesser than 10 bacilli per 100 fields. The time needed by fluorescent microscopy to screen a slide for declaring it as negative was 3 minutes when compared to 7 minutes with the Ziehl-Neelsen procedure. The results obtained by one technique are highly reproducible by the other technique .

Fluorescent microscopy detects more paucibacillary cases than conventional microscopy, and it also reduces the required screening time by 50% **.**

Singh NP and Parija SC [11] compared light microscopy with fluorescent microscopy by studying a total of 2600 sputum specimens in clinically suspected cases of pulmonary tuberculosis . On the whole , 1104 samples were positive for Acid Fast Bacilli (AFB) by either of the techniques . Ziehl Neelsen picked up a total of 975 (37.5 %) positive samples and Auramine staining picked up an additional 129(4.96%) positive samples. They concluded that fluorescent microscopy using auramine staining is a better method in terms of greater sensitivity as well as time consumption .

C. Padmapriyadarsini , G. Narendran , and Soumya Swaminathan et al,[12] suggested that some of the methods to improve the sensitivity of sputum smear microscopy in HIV – TB co infected individuals include the use of fluorescent microscopy and various sputum concenteration and processing techniques. Also the increased cost of fluorescent microscope limits its use in resource limited settings. The recent use of light emitting diode bulbs in fluorescent microscopes at much cheaper costs and its superiority over other techniques offers a promising

future for sputum microscopy. On any account, because sputum smear is the primary method for detection of TB in many resource constrained settings, a considerable number of smear-negative individuals often remain undetected or get delayed anti-tuberculous therapy. This hurdle can also be overcome by using more sensitive techniques.

R. KUMAR, MITHLESH AGRAWAL and M. PRASAD [13] in 1979 compared ziehl neelsen staining, fluorescent microscopy and mycobacterial culture. They studied 574 patients , out of which 265(46%), 275(48%) and 284(49%) were positive for acid fast bacilli by ziehl neelsen staining, fluorescent microscopy and mycobacterial culture. They also correlated the extent of disease radiologically (Shanks and Peter Kerley in 1973) and different bacteriological techniques. In cases with minimal involvement , 33.5%, 37.1% and 36.5% cases were positive for tubercle bacilli by ziehl neelsen staining, fluorescent microscopy and mycobacterial culture respectively. In cases with no cavitation , 34.8%, 36.4% and 38.0% cases proved to be positive by ziehl neelsen staining, fluorescent microscopy and mycobacterial culture respectively.

Steingart et al.[14] searched many databases , contacted many experts and did a systematic review of 18 studies which he identified . The various databases searched include **Embase (from 1974 to 2004),**

PubMed (from 1950 to May 2005) , BIOSIS (from 1969 to November 2004) and Web of Science (from 1945 to 2004). They also searched the two main journals devoted solely to tuberculosis , ***The International Journal of Tuberculosis and Lung Disease*** **(1997 to 2005) and** ***The Indian Journal of Tuberculosis*** **(1953 to 2004) ,** for articles regarding sputum microscopy .The studies which were excluded are those wherein : (1) microscopy was done on samples other than sputum , (2) use of smear microscopic methods to identify non tuberculous mycobacteria , (3) use of sputum samples to evaluate the response to anti-tuberculous therapy, (4) those studies which mainly concentrate on economic issues such as cost - effectiveness ,(5) case reports and case series , and (6) review articles . Incremental yield , sensitivity and specificity were the variables considered as the outcomes of interest. The quality of various studies was also assessed . They reported that fluorescent microscopy using Auramine O staining of sputum smears provides the same specificity but an increase in sensitivity (mean improvement of 10%), when compared with light microscopy using Ziehl Neelsen staining. Finally they inferred that before Fluorescent Microscopy can be implemented as a routine in under developed countries , several complex issues have to be addressed and practical difficulties need to be solved . These include , (1) feasibility and maintenance of fluorescent microscopes in labs with irregular and inadequate electric supply, (2)

minimal human and financial resources, and inadequate training of the laboratory personnel ; (3) the lack of globally accepted external quality assessment standards for blinded re checking of fluorescent smears; (4) the stability of reagents used for fluorescent microscopy at the field level (such as extremes of temperature and optimum environment). These obstacles should be overcome by operational research . Only then , policy changes can be made .

Masood Ziaee , Mohammad Namaei , Majid Khazaei , Ghodsieh Azarkar [15] from Iran studied 920 consecutive patients suspected of having pulmonary TB, referred to TB laboratory, provincial office of health care, Birjand University of Medical Sciences, Iran . They collected a total of 2760 sputum specimens from them between April 1996 and April 2004. All samples were smeared and stained using both Ziehl Neelsen and auramine phenol methods according to WHO recommendations . Two independent experts examined the smears microscopically. All smears that were positive by fluorescent microscopy were re - stained by Ziehl Neelsen technique for confirmation. The sensitivity of Ziehl Neelsen staining was also evaluated in different contamination conditions. A total of 102 out of 920 study subjects had pulmonary TB(as confirmed by culture) , out of which 68 (66.66%) patients were smear positive by either staining method while others were smear negative. The proportion of positive smears detected was 51% and

57% for the Ziehl Neelsen and auramine phenol staining methods, respectively. The sensitivity, specificity, positive predictive value and negative predictive value were 51% ,100% ,100% , 94% for the Ziehl Neelsen method and 57% ,100% ,100 % , 95 % for auramine phenol staining methods, respectively. The agreement in grading between the two methods was 93.2%. Ziehl Neelsen method missed 16 (27.6%) of the 58 slides found positive by the auramine phenol method while auramine phenol method missed only 10 (19.2%) of the 52 slides found positive by the Ziehl Neelsen method. The performance of the Ziehl Neelsen method and auramine phenol method were evaluated using a combination of smear result and clinical picture of patients as the **"gold standard."** They also compared the sensitivity of Ziehl Neelsen staining in different contamination conditions. Results showed that in 1+, 2+ and 3+ contamination, the sensitivity of Ziehl Neelsen staining was 70%, 67%, , and 83%, respectively. This study also showed a relationship between Ziehl Neelsen missed positive smears and density of bacilli on fluorescent microscopy stained smears. This is in agreement with other studies. The employment of fluorescent microscopy significantly increases the diagnostic value of the sputum smear microscopy especially in pauci bacillary patients who are likely to be missed on Ziehl Neelsen stained smears.

Habeenzu et al[16] study revealed that among the 488 specimens, 152 were positive for acid-fast bacilli using fluorescent microscopy while only 66 were positive by Ziehl Neelsen method .

Mustafa Ulukanligil et al [17] also compared the sensitivity of these two techniques and they found higher sensitivity by fluorescent microscopy (85.3%) than Ziehl Neelsen (67.6%) in HIV patients. False positive results are a problem in diagnostic AFB smear. False positive results should be highly suspected , when the obtained specimen is from a person receiving anti tuberculosis drugs and also when specimen is bloody. Therefore, it is recommended that positive or doubtful fluorochrome staining smear results are better to be confirmed by Ziehl Neelsen stained smear or a second examiner .

N.Selvakumar et al conducted a study in Tuberculosis Research Center, Chennai[18] to study the detection rates using fluorescent microscopy and Ziehl Neelsen staining to stain tubercle bacilli in sputum samples stored in cetylpyridinium chloride (CPC) solution . In most circumstances, sputum samples cannot be immediately transported to laboratories. When there is a delay in transportation of sample , the rate of isolation of organism of mycobacteria from the specimen is considerably reduced . To overcome this problem , sputum samples need to be preserved

in an effective manner using an optimum preservative which does not affect the isolation rate of the organism . Hence in this study sputum samples were stored in cetylpyridinium chloride (CPC) solution and then smears were prepared , stained and studied . Totally 988 sputum samples were collected in cetylpyridinium chloride (CPC) solution and 2 smears were prepared from each sample. The statistical analysis was done using Mc nemar ' s paired test. Among these , 377 samples were positive using fluorescent microscopy . Out of these 377 samples , 166 (44%) were negative using Ziehl Neelsen technique . **There were more samples which were culture-positive, but smear negative while using Ziehl Neelsen technique (52.7%) than while using auramine phenol (30%) .** In another, separate study , totally 104 sputum samples were collected from patients with chest symptoms indicative of pulmonary tuberculosis. Direct smears were prepared immediately and stained by Ziehl Neelsen technique . Cetylpyridinium chloride (CPC) was added to these samples and stored in necessary conditions for 7 days, following which sputum smears were prepared and stained using Ziehl Neelsen. Totally 52 specimens were positive by Ziehl Neelsen technique when stained directly after collection. The smears prepared from preserved samples showed that only 35 were positive by Ziehl Neelsen technique . They found that detection of tubercle bacilli in sputum samples preserved in cetylpyridinium chloride is

significantly decreased while using Ziehl Neelsen staining , whereas fluorescent microscopy results were not much affected by this preservative technique . There was no data on the fact whether cetylpyridinium chloride (CPC) could affect the cell wall integrity of Mycobacterium tuberculosis . It was concluded that the liquefaction of sputum due to dilution in cetylpyridinium chloride (CPC) solution might have led to the drastic reduction in detection rates.

Today , several automated culture systems and molecular techniques have come into practice for the diagnosis of tuberculosis . All these new modalities of diagnosis have reduced the time required for the detection of acid fast bacilli . But these are quite expensive and cannot implemented under proramme conditions in highly endemic low income countries. Thus there is an immediate need to improve the sensitivity of sputum smear microscopy until newer cost effective alternatives come up. To attain this objective , various techniques have been tried individually with varying degrees of success (10 – 23 % increase in sensitivity). Some of these techniques include concentration of sputum , sedimentation techniques and fluorescent microscopy . These individual techniques when combined could increase the sensitivity of sputum smear microscopy significantly . This was done by Hooja et al in 2008 - 09 by concentrating the sputum specimens and studying them using fluorescent microscope.

Hooja et al[19] conducted a study in Jaipur , India from January 2008 to December 2009 on various clinical specimens obtained from patients suspected to have tuberculosis .They wanted to evaluate the increase in efficacy of AFB microscopy when fluorescent microscopy is used to detect Acid Fast Bacilli over concentrated samples (concentration done by Petroff 's method) when compared with Ziehl Neelsen technique . A total of 393 samples were collected for this study which included sputum , pleural fluid , broncho alveolar lavage , tracheal aspirate , urine , cerebro spinal fluid , lymph node aspirate and ascitic fluid . These samples were centrifuged and subjected to concentration by Petroff 's method . They prepared two sets of smears from the samples , one from direct specimen and other from concentrated specimen . The concentrated specimens were also inoculated on Lowenstein Jensen medium . A total of ten specimens which were identified as contaminated on culture. These samples were eliminated from the study and the statistical analysis was carried out on the remaining 383 samples .

The results were as follows :

ZIEHL NEELSEN TECHNIQUE

	DIRECT SMEAR	**CONCENTRATED SMEAR**
SMEAR POSITIVITY FOR AFB (OVERALL)	**20.10% (77 / 383)**	**22.45 % (86 / 383)**
PULMONARY SPECIMENS	29.92% (76 / 254)	-
EXTRA PULMONARY SPECIMENS	0.77% (1 / 129)	-
SENSITIVITY	55.55%	62.22%
SPECIFICITY	99.19%	99.19%
EFFICIENCY	83.81%	84.85%

The sensitivity and specificity were obtained by comparing against mycobacterial culture in LJ medium as the reference standard.

FLUORESCENT MICROSCOPY

	DIRECT SMEAR	**CONCENTRATED SMEAR**
SMEAR POSITIVITY FOR AFB (OVERALL)	**25.84% (99 / 383)**	**29.76 % (114 / 383)**
PULMONARY SPECIMENS	38.18% (97 / 254)	-
EXTRA PULMONARY SPECIMENS	1.55% (2 / 129)	-
SENSITIVITY	71.85%	82.96%
SPECIFICITY	99.19%	99.19%
EFFICIENCY	89.55%	91.64%

Kivihya - Ndugga LE et al[20] conducted a cross sectional study in Kenya on 1398 patients suspected to have tuberculosis. They wanted to evaluate the cost effectiveness and efficiency of conventional Ziehl - Neelsen (ZN) and fluorescent microscopy (FM).They used mycobacterial culture in LJ medium as gold standard. A chest X-ray was also taken. Fluorescent Microscopy (FM) had a greater sensitivity than Ziehl - Neelsen (ZN) method ($P < 0.001$). The performance efficacy of Fluorescent Microscopy was not affected by the patient's HIV status. The cost per each smear - positive patient , was 40.30 US dollars for Fluorescent Microscopy (FM) on two specimens compared to 57.70 US dollars for Ziehl - Neelsen (ZN) on three specimens. The Fluorescent Microscopy (FM) method is more efficient and cost-effective and shortens the diagnostic process.

WHO POLICY STATEMENT (MARCH 2010) ON LED MICROSCOPES[21]

Fluorescent microscopes with Light-emitting diodes (LED) were developed to provide the advantages of fluorescent microscopy without the associated mammoth expenditure of conventional fluorescent microscopes. In September 2009, the efficiency of LED microscopes in sputum smear microscopy for detection of tubercle bacilli was assessed by the World Health Organization (WHO), based on appropriate international standards. This was achieved in three steps. First, the WHO experts did a systematic

review and meta-analysis of the published and also the unpublished data of the standard diagnostic methods . Second, an expert group was convened to evaluate the strength of the evidence and provide operational and logistical recommendations for using LED microscopy in national level control programmes for TB . This also helped to identify gaps to be addressed by future research . As the final step, a draft of the recommendations was presented for endorsement to the " **WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) "**. This team also assessed the precision and the various effects of new diagnostic methods of TB on patients health and public health. The results showed that the accuracy of LED microscopy was equivalent to that of international reference standards. Also it was found to be more sensitive than conventional Ziehl-Neelsen microscopy and it had qualitative, operational and cost advantages over both conventional fluorescence and Ziehl- Neelsen microscopy. Following results were obtained. When compared with culture as reference standard, LED microscopy showed 84 % sensitivity and 98 % specificity. When a microscopic reference standard was used, LED microscopy showed 93% sensitivity and 99% specificity. They reported a significant increase in sensitivity when direct sputum smears were used instead of concentrated sputum smears (89% and 73%, respectively). On comparing with individual microscopic techniques, (i) LED microscopy vs

Ziehl - Neelsen microscopy : a 6% increase in sensitivity with no appreciable loss in specificity was observed, (ii) LED microscopy vs conventional fluorescent microscopy : a 5 % increase in sensitivity and 1 % increase in specificity was observed while using LED microscopy. On the basis of these studies, the World Health Organisation has recommended that conventional fluorescenct microscopy must be replaced by LED microscopy, and also that LED microscopy should step in as an alternative technique for conventional Ziehl-Neelsen light microscopy. The switch over to LED microscopy should be carefully brought about at national level, with the kind of LED technology that stays on par with specifications set by WHO. Countries that switch over to LED microscopy must train their laboratory personnel , validate the technique, introduce proper quality assurance methods and monitor the effect of LED microscopy on case detection rates of TB and treatment outcomes.

LED microscopy was introduced mainly to give developing and under developed countries a chance to enjoy the advantages of fluorescent microscopy. Fluorescent microscopes use conventional mercury vapour lamps as their excitatory light source. These lamps have a short half-life and a risk of releasing potentially hazardous products if broken. They also require dark room for optimum performance. LED microscopes need lesser power. They run on batteries ; furthermore , the bulbs have a longer life

span and they do not have the risk of releasing hazardous products or UV radiation if broken . LED microscopes are said to perform equally well in a bright room . These properties make LED microscopy easy to install and use in resource-limited settings .

Marais BJ et al in Desmond Tutu TB Center, Cape town, South Africa[22] collected sputum samples from 221 persons with chest symptoms suggestive of TB, stained them with auramine O and studied the smears using two excitatory sources, namely mercury vapour lamps and LED lamps. These smears were re stained by Ziehl Neelsen technique and re examined using light microscope . They used mycobacterial culture as the reference gold standard. While using LED microscopes, the sensitivity was 84.7% and the specificity was 98.9%. Sensitivity and specificity was 73.6% and 99.8% respectively for conventional mercury vapour lamps. For light microscopes, sensitivity was 61.1% and specificity was 98.9%.

A similar stud**y** was conducted by **Habtamu et al**[23] , in Kilimnjaro, Tanzania to determine the value of LED microscopy in diagnosing tuberculosis on bleach treated and direct sputum smears. Four smears were prepared from each specimen: conventional Ziehl-Neelsen (ZN), direct auramine, bleach centrifugation and bleach short sedimentation auramine smears. Of the 267 sputum samples examined, respectively 29%, 23%, 28%

and 18% were acid-fast bacilli (AFB) positive by the bleach centrifugation, bleach short sedimentation, direct auramine and ZN methods. Bleach centrifugation identified 11% more positives than ZN microscopy ($P < 0.001$), but was not superior to the direct auramine method ($P=0.46$), which yielded 10% more positives than ZN microscopy ($P < 0.001$). They concluded that LED microscopy on direct sputum smears was more rapid, easy and effective.

Shenai S, et al in Hinduja hospital , Mumbai [24] evaluated the use of LED fluorescent microscopy in a laboratory experienced in Ziehl Neelsen microscopy but unfamiliar with Fluorescent Microscopy. Both these techniques were evaluated in parallel and compared with mycobacterial culture as the reference standard. In this study , a total of 1357 sputum samples along with 917 extra pulmonary specimens were examined .The Sensitivity and specificity was 78.3% and 92.0% for sputum samples and 34.0% sensitivity and 88.8% specificity was obtained for extra-pulmonary specimens for LED fluorescent microscopy when compared against mycobacterial culture. The mean time per smear examination was 2.48 min for Ziehl Neelsen microscopy vs. 1.41 min for LED fluorescent microscopy . They concluded that though LED fluorescent microscopy has significant benefits , at the present scenario its implementation and validation has many practical difficulties which could

hamper its optimum performance. The standardization of operating procedures and adequate training are required to maximize accuracy.

G. Torrea et al[25] evaluated the efficiency of fluorescence microscopy using Fluoreslen S by a blinded rechecking trial in Nairobi, Kenya **.** The high installation rates and problems associated with the usage and maintenance of fluorescent microscopy equipment are the major reasons why they are rarely used for sputum smear microscopy in high - prevalence countries. The Fluoreslen S system converts standard light microscopes into fluorescence microscopes. It features an external high intensity halogen light source connected via a fibre optic cable to a microscope objective with in-built filters. This system is cheap, can be operated very easily and the halogen bulbs have a much longer life-span . For evaluating this system , three public health facilities in Nairobi, Kenya, were selected for their high workload (500–1500 smears per month).

A total of 46,354 smears were examined. Out of this , 25,250 by Ziehl Neelsen (ZN) and 21,104 by Fluorescent Microscopy(FM). The average prevalence of positives was 19.5% for Ziehl Neelsen ZN and 23.0% for Fluorescent Microscopy (FM.) Fluorescent Microscopy (FM) detected on average 18% positives more than Ziehl Neelsen ZN, or A total of 2807 Ziehl Neelsen (ZN) and 1982 Fluorescent Microscopy(FM) smears were

rechecked. Error rates of Ziehl Neelsen (ZN) and (FM) Fluorescent Microscopy were low and similar, considering all centres separately and together. Overall, false-negatives reached 1.8% by Ziehl Neelsen (ZN) and 2.6% by Fluorescent Microscopy (FM)(non-significant).

The case detection rates of tuberculosis are very much below the desired targets in most of the developing and under developed countries. The standard approach of sputum smear microscopy consists of collection of sputum over two or three days and examining the smears by conventional light microscopy using Ziehl Neelsen method which is a time -consuming as well as an insensitive technique. **Cattamanchi A et al** [26] conducted a study in Kampala , Uganda to find out whether two different approaches when combined can cause a significant increase in case detection rates of pulmonary tuberculosis. These two approaches include using single sputum specimen and screening it by LED fluorescent microscopy . Patients with productive cough > 2 weeks were admitted . Two sputum samples were collected from each patient one spot sample and one early morning sample .The study consisted of two parts . The first part compared the efficacy based on the number of sputum samples collected (single sample versus two samples) . The second part compared the diagnostic accuracy based on the type of microscopy (light microscopy versus fluorescent microscopy) . Mycobacterial culture was used as a

reference standard . On the whole , 233 out of 464 (50%) cases were positive for TB by culture . There was not much difference in sensitivity between usage of single - specimen and two - specimen when smears were examined with light microscopy. The technique of LED fluorescent microscopy had a greater sensitivity when compared to conventional light microscopy with both the single-sample (61% versus 55%) and two-sample strategies (64% versus 56%). Similar findings were observed among the subset of HIV-infected patients . In countries with low-income and high burden of Tuberculosis , using a single - sputum and LED fluorescent microscopy, either individually or in combination , would significantly increase the detection of smear-positive cases of Tuberculosis.

At present , Fluorescent microscopes are provided to the state designated Intermediate Reference Laboratories under Revised National Tuberculosis Control programme (RNTCP). There the use of fluorescence microscopy is linked to the culture and Drug Sensitivity Testing activities .

MATERIALS AND METHODS

METHODOLOGY

PATIENTS

This study was conducted at **GOVT. STANLEY HOSPITAL**. For this study, chest symptomatics referred from various departments to **RNTCP cell** of Govt. Stanley medical college for sputum AFB smear microscopy were selected. Patients were selected based on their history and physical examination. All the patients were enrolled in the study after fulfillment of inclusion and exclusion Criteria and getting appropriate consent.

STUDY PERIOD : 6 months from APRIL 2012 TO SEPTEMBER 2012

The patients were advised routine two sputum smear samples for (Ziehl Neelsen) microscopy as per RNTCP guidelines. From the home collection specimen, two smears were prepared, one for Ziehl Neelsen microscopy and the other for fluorescence microscopy. The technicians were not allowed to cross check the results to avoid bias. Standard stastical methods were used to assess the correlation between the variables.

INCLUSION CRITERIA

All chest symptomatics referred for sputum smear Acid Fast Bacilli as per RNTCP criteria

1. Chronic cough >2weeks
2. Evening rise of temperature
3. Unexplained weight loss

EXCLUSION CRITERIA

1. ATT treatment failure patients
2. Relapse of pulmonary tuberculosis
3. ATT defaulters
4. Age < 13 years

SUPPORTIVE TESTS

In addition to sputum smear microscopy , a chest X- ray PA view of the patient was also obtained and the findings were noted.

PROCEDURE

Preparation of sputum smear

1. A glass slide should be taken and cleaned.
2. A small amount of sample is taken using a loop and smeared on the slide by spreading slowly such that it forms a thin film measuring 1cm in diameter.

3. Care should be taken that sputum droplets do not get splashed into the surroundings.
4. The smear should get dried adequately.

ZIEHL NEELSEN METHOD

REAGENTS

1. Carbol fuschin
2. 25% Sulphuric Acid
3. Methylene Blue
4. Distilled Water

PROCEDURE

1. Add carbol fuschin such that it covers the slide.
2. Heat the smear from the under aspect of the slide for 7-8 mins so that steam arises. Take care the smear should not boil.
3. Wash with distilled water and decolourise with 25% sulphuric acid for 2 minutes.
4. Wash with distilled water again and add counterstain – methylene blue.
5. Wait for 30 seconds, wash again and dry the slide.
6. Observe under 100x oil immersion using light microscope.

RESULTS :

Acid fast bacilli are observed as as slender pink rods against a blue background.

GRADING

NO. OF AFB	PER	GRADE
>10	single oil immersion field in atleast 20 fields	**3 +**
1-10	single oil immersion field in atleast 50 fields	**2 +**
10-99	PER 100 oil immersion fields	**1 +**
1-9	PER 100 oil immersion fields	scanty / record the exact no. of bacilli

FLUORESCENT MICROSCOPY

In fluorescent microscopy, the mycobacteria are identified by staining with a fluorochrome named Auramine O. The mycobacteria take up the stain because of the strong affinity of the fluorochrome for the mycolic acid present in their cell wall. Here Potassium permanganate is used as counter-stain and it helps prevent non-specific fluorescence. With auramine staining, the bacilli are identified as luminous thin yellow rods prominently against

a dark background . A mercury vapour lamp is used as a source of light and by means of suitable filter , only light rays of shorter wave lengths are allowed to emerge and these rays are used for microscopy. The fluorochromes absorb these light rays with shorter wavelengths and emit light rays with longer wavelengths . The condenser of the microscope is made of quartz which will not absorb ultra-violet rays.

REAGENTS

Auramine O stain with phenol commercial preparation was used

1. Primary stain- Auramine O stain with phenol
2. Decolouriser - concenterated Hydrochloric acid in 70% ethanol
3. Counter stain - Potassium permanganate

PROCEDURE

1. The sputum smear was heat fixed and stained with the phenolic Auramine stain and kept for 15 minutes.
2. Wash the slide with distilled water.
3. The sputum smear is decolourised for two minutes by covering it with 0.5% hydrochloric acid in 70% ethanol .
4. The slide is washed with distilled water .
5. The smear is counterstained by covering it for two minutes with 0.5% aqueous potassium permanganate.

6. The slide is washed with distilled water , air dried and examined under low power (200x) and then confirm under oil immersion (1000x).

RESULTS :

Acid fast bacilli appear as greenish yellow fluorescent rods against a darker back ground.

PRECAUTIONS

1. Avoid making thick smears.
2. Strong counter staining should be avoided because it prevents adequate decolourisation and also masks the identification of acid fast bacteria , if present .
3. The smears stained with Fluorescent dyes are to be read within 24 hours of staining because of fading.
4. Stained smears have a tendency to fade on exposure to light. The slides are to be stored in the slide box or wrapped in brown or black paper to avoid exposure to light.
5. Auramine phenol preparation should be stored in a dark brown bottle. The stain should not be kept for more than 3 weeks.
6. After making smear, burn and dispose the broom - stick or flame wire loop thoroughly using side burner prior to re-use.

GRADING

AURAMINE O FLUORESCENT STAINING GRADING (USING 20 OR 25X OBJECTIVE AND 10X EYE PIECE)	**REPORTING /GRADING**
>100 AFB / field after examination of 20 fields	3+
11-100 AFB / field after examination of 50 fields	2+
1-10 AFB / field after examination of 100 fields	1+
1-3 AFB / 100 fields	Doubtful positive/repeat

For the purpose of uniformity while using a fluorescent microscope and to avoid errors in reporting , WHO has devised the method of using "magnification correction factor". The value obtained by this method gives an apoproximate estimate of the number of acid-fast bacilli that will be detected if the same smear is observed under 1000 X magnification in an ordinary light microscopy after carbol fuchsin staining. In a fluorescent microscope, when a 40 X objective is used , the number of organisms seen under FM is divided by a magnification correction factor of 10.

MAGNIFICATION CORRECTION FACTOR

S.No.	FM OBJECTIVE MAGNIFICATION (POWER) *	MAGNIFICATION CORRECTION FACTOR
1	20 X	10
2	25 X	10
3	40 X	5
4	45 X	4
5	63 X	2

To obtain the comparative grading, divide the observed count of AFB under the FM objective with this factor before grading.

ANALYSIS OF RESULTS

102 patients with chest symptoms as per the RNTCP criteria participated in this study. The results of the study were as follows :

TABLE : 1

AVERAGE AGE OF THE CHEST SYMPTOMATICS STUDIED

S.No.	N	Minimum age	Maximum age	Mean	Standard Deviation
1	102	16	74	42.18	13.968

The minimum age of the participants was 16 years

and the maximum age of the participants was 74 years .

The mean age was 42.18 and the standard deviation was 13.968 .

TABLE : 2

AGE DISTRIBUTION OF THE CHEST SYMPTOMATICS STUDIED

AGE	FREQUENCY
16. to 30 YRS	23
31. to 45 YRS	42
46. to 60 YRS	24
61 AND ABOVE	13

Maximum number of patients (42) were found in the age group of 31 to 45 years and Minimum number of patients (13) were found in the age group of more than 61 years.

AGE DISTRIBUTION OF THE CHEST SYMPTOMATICS STUDIED

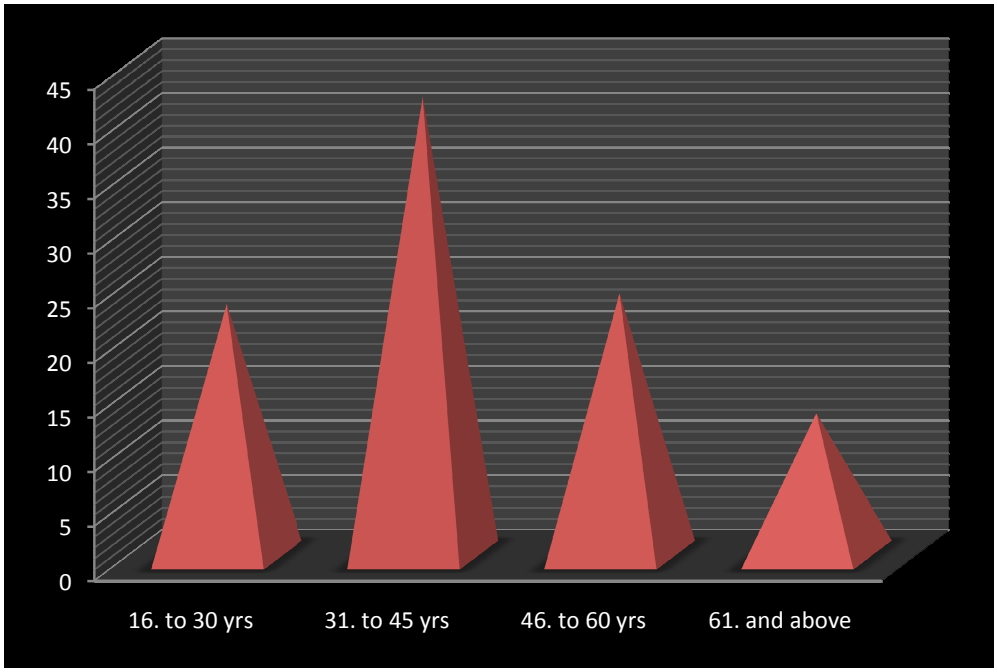

Figure - 1

TABLE : 3

AGE DISTRIBUTION OF THE PATIENTS WHOSE SPUTUM WAS POSITIVE FOR AFB

AGE GROUP	FREQUENCY	IN PERCENTAGE (%)
16 – 30 YRS	**12**	**21.4**
31 – 45 YRS	**28**	**50.0**
46 – 60 YRS	**12**	**12.0**
61 YRS AND ABOVE	**4**	**7.1**

Among the 56 patients whose sputum was positive for AFB ,

MAXIMUM NUMBER(50%) OF PATIENTS WERE IN THE

AGE GROUP OF 31 – 45 YEARS

MINIMUM NUMBER (50%) OF PATIENTS WERE IN THE

AGE GROUP OF MORE THAN 61 YEARS

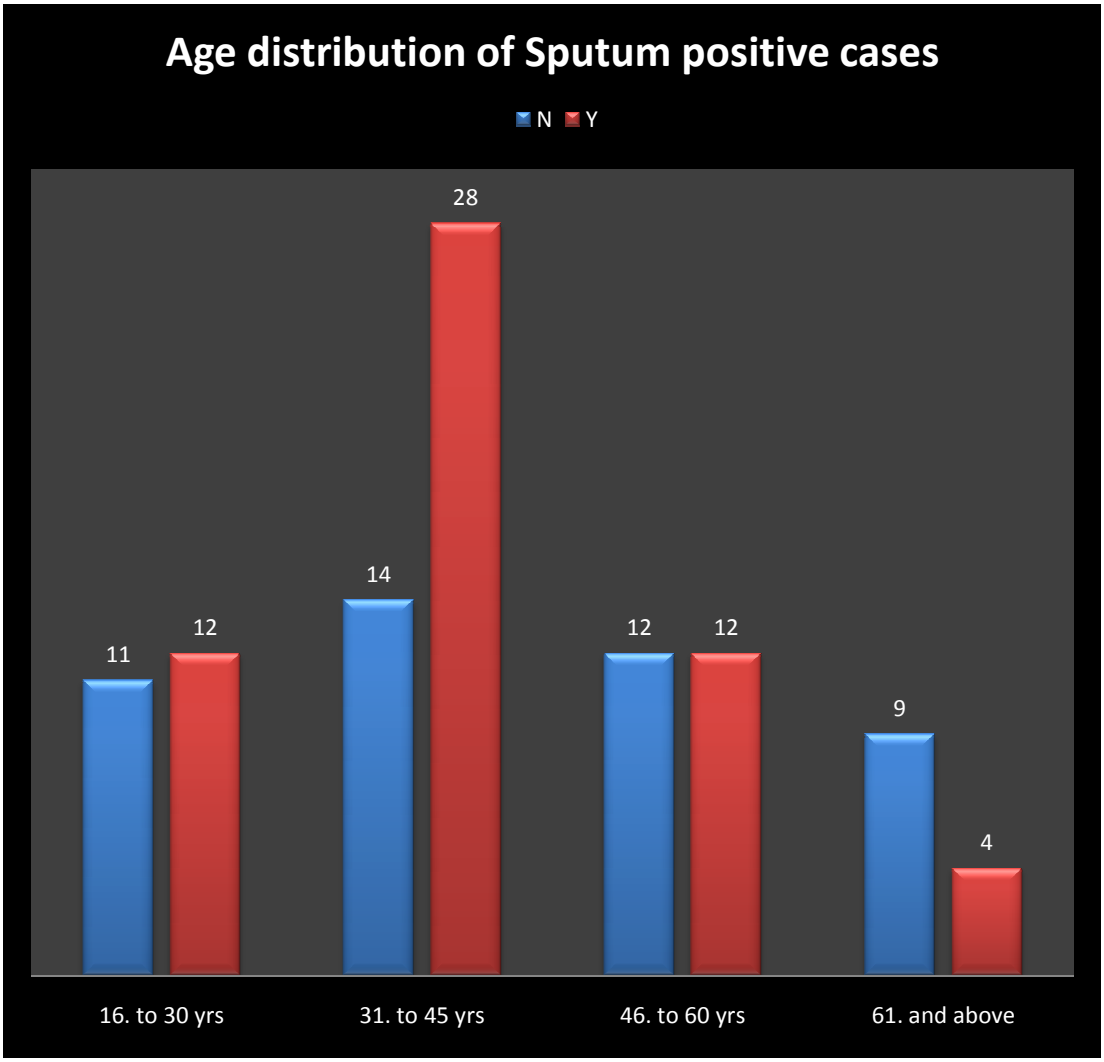
Age distribution of Sputum positive cases
N
Y
11
12
14
28
12
12
9
4
16. to 30 yrs
31. to 45 yrs
46. to 60 yrs
61. and above

Figure - 2

TABLE : 4

GENDER DISTRIBUTION OF THE CHEST SYMPTOMATICS STUDIED

SEX	FREQUENCY	IN PERCENTAGE (%)
FEMALES	**21**	**20.6**
MALES	**81**	**79.4**
TOTAL	**102**	**100.0**

AMONG THE 102 STUDY PPARTICIPANTS , 81 (20.6 %) WERE MALES AND 21 (79.4 %) WERE FEMALES.

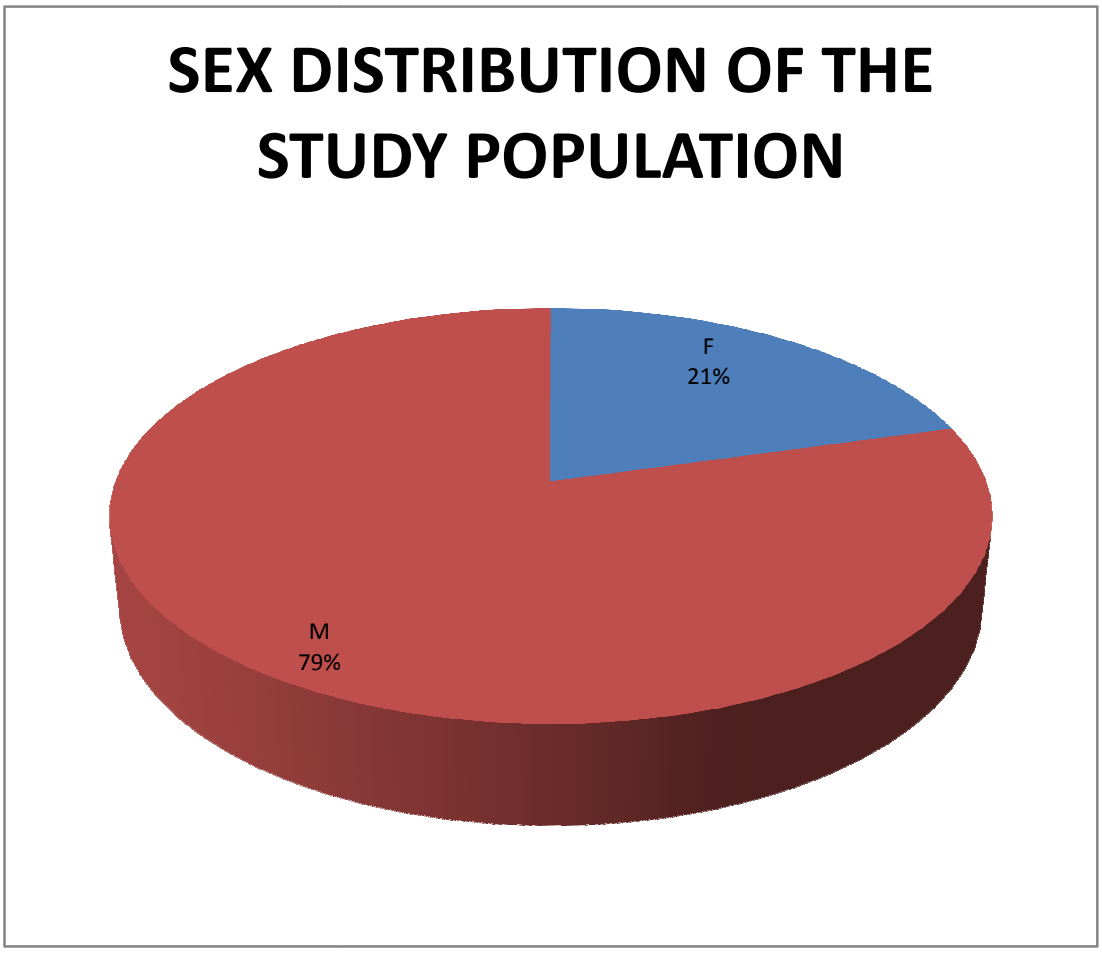

Figure - 3

TABLE : 5

GENDER DISTRIBUTION OF THE PATIENTS WHOSE SPUTUM WAS POSITIVE FOR AFB

SEX	FREQUENCY	IN PERCENTAGE (%)
FEMALES	**13**	**23.2**
MALES	**43**	**76.8**
TOTAL	**56**	**100.0**

AMONG THE 56 PATIENTS POSITIVE FOR AFB , 43 (76.8 %) WERE MALES AND 13 (23.2 %) WERE FEMALES.

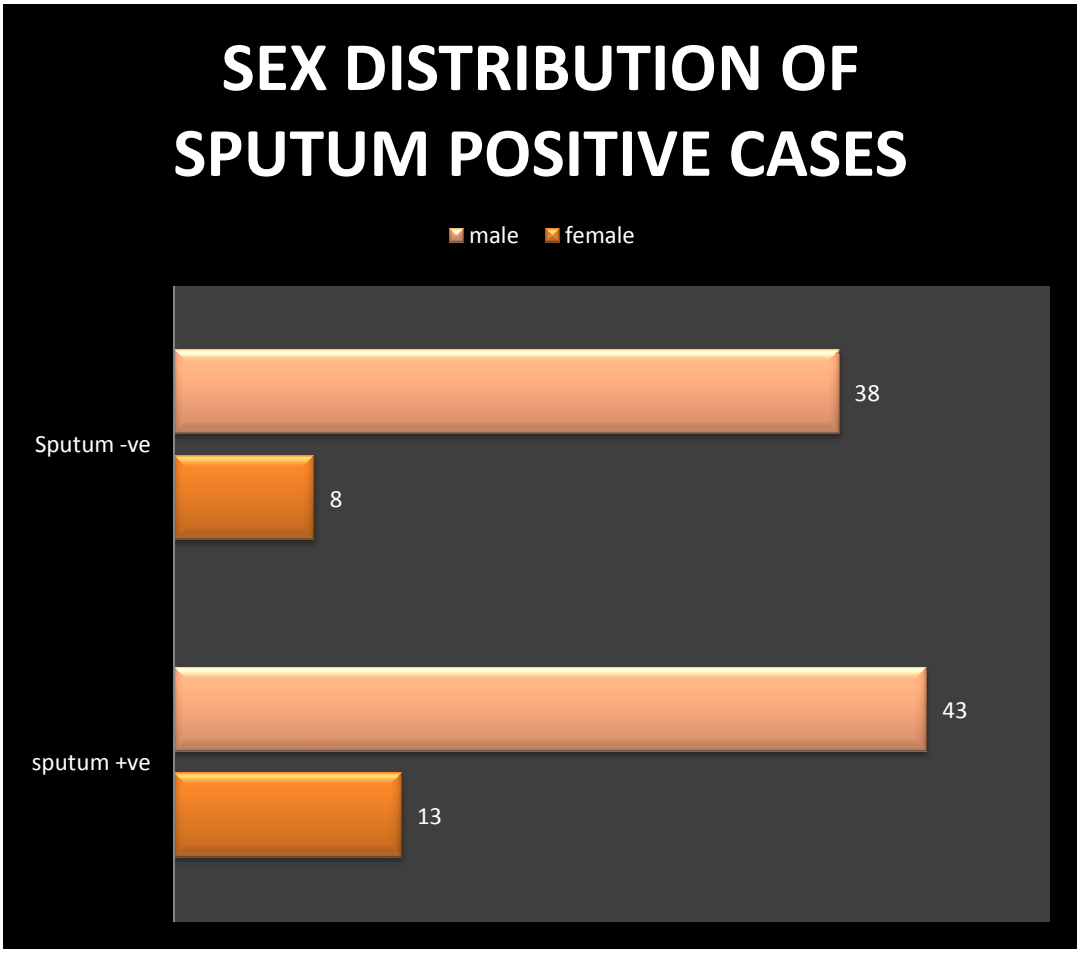
SEX DISTRIBUTION OF SPUTUM POSITIVE CASES
male
female
Sputum -ve
38
8
sputum +ve
43
13

Figure - 4

TABLE : 6

CLINICAL PRESENTATION OF PULMONARY TB CASES

SYMPTOM	FREQUENCY	PERCENTAGE
1.COUGH	46	82.1
2.HAEMOPTYSIS	12	21.4
3.BREATHLESSNESS	25	44.6
4.FEVER	44	78.6
5.LOSS OF APPETITE	19	33.9
6.LOSS OF WEIGHT	37	66.1
7.CHEST PAIN	10	17.9
8.LYMPHADENOPATHY	7	12.5

The most common symptom of sputum positive tuberculosis was found to be cough (82.1 %) followed by fever (78.6 %) and loss of weight (66.1 %) . Other symptoms were breathlessness (44.6 %) , loss of appetite (33.9 %) , haemoptysis (21.4 %) , chest pain(17.9%) and rarely lymphadenopathy (12.5 %).

CLINICAL PRESENTATION OF SPUTUM POSITIVE PULMONARY TB CASES

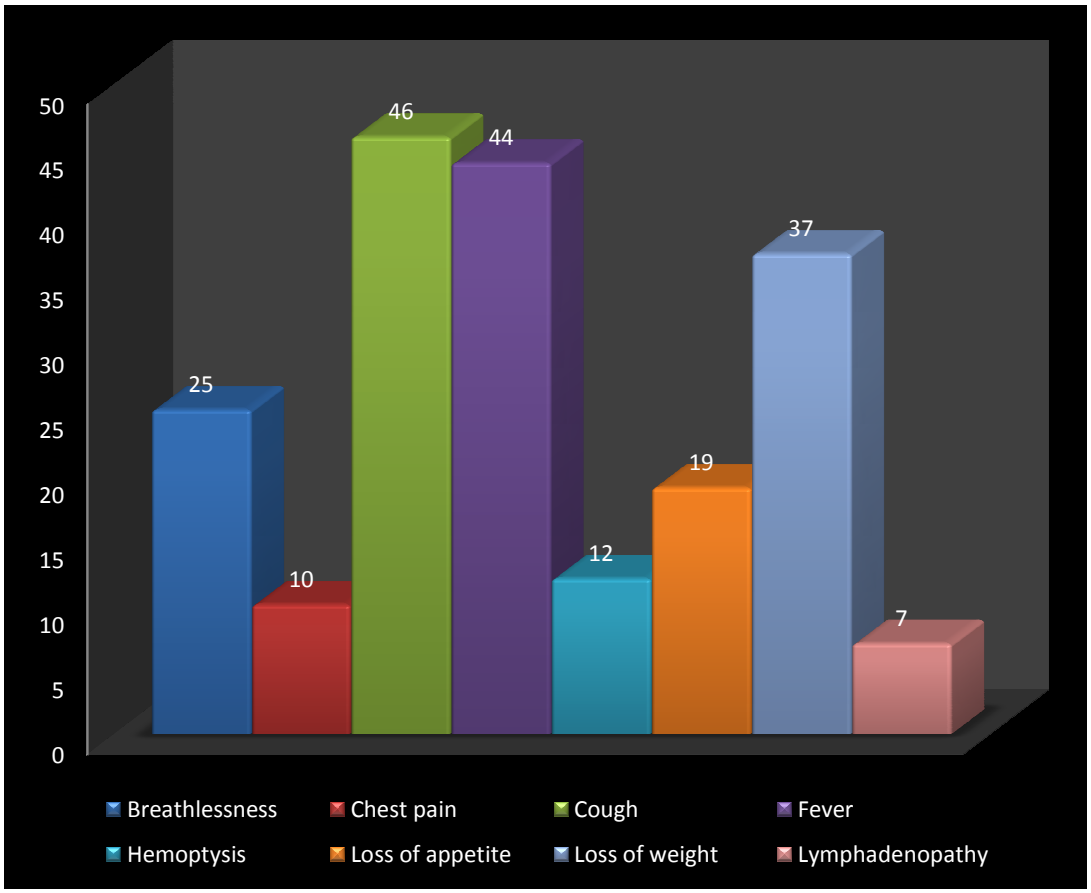

Figure - 5

TABLE : 7

IMAGING FINDINGS IN SPUTUM POSITIVE PULMONARY TB

FINDING	FREQUENCY	PERCENTAGE
1.INFILTRATES	29	51.8
2.CONSOLIDATION	20	35.7
3.CAVITY	5	8.9
4.MILIARY TB	1	1.7
5.COLLAPSE	1	1.7

Among the 56 patients with sputum positive pulmonary tuberculosis , X – ray chest showed infiltrates in the form of small heterogenous opacities in 51.8 % of the patients followed closely by tuberculous consolidation in 35.7 % of the patients . Findings like cavity was found in 5 (8.9 %) patients and patterns of collapse (1.7%) and miliary TB (1.7) were found each in only one patient.

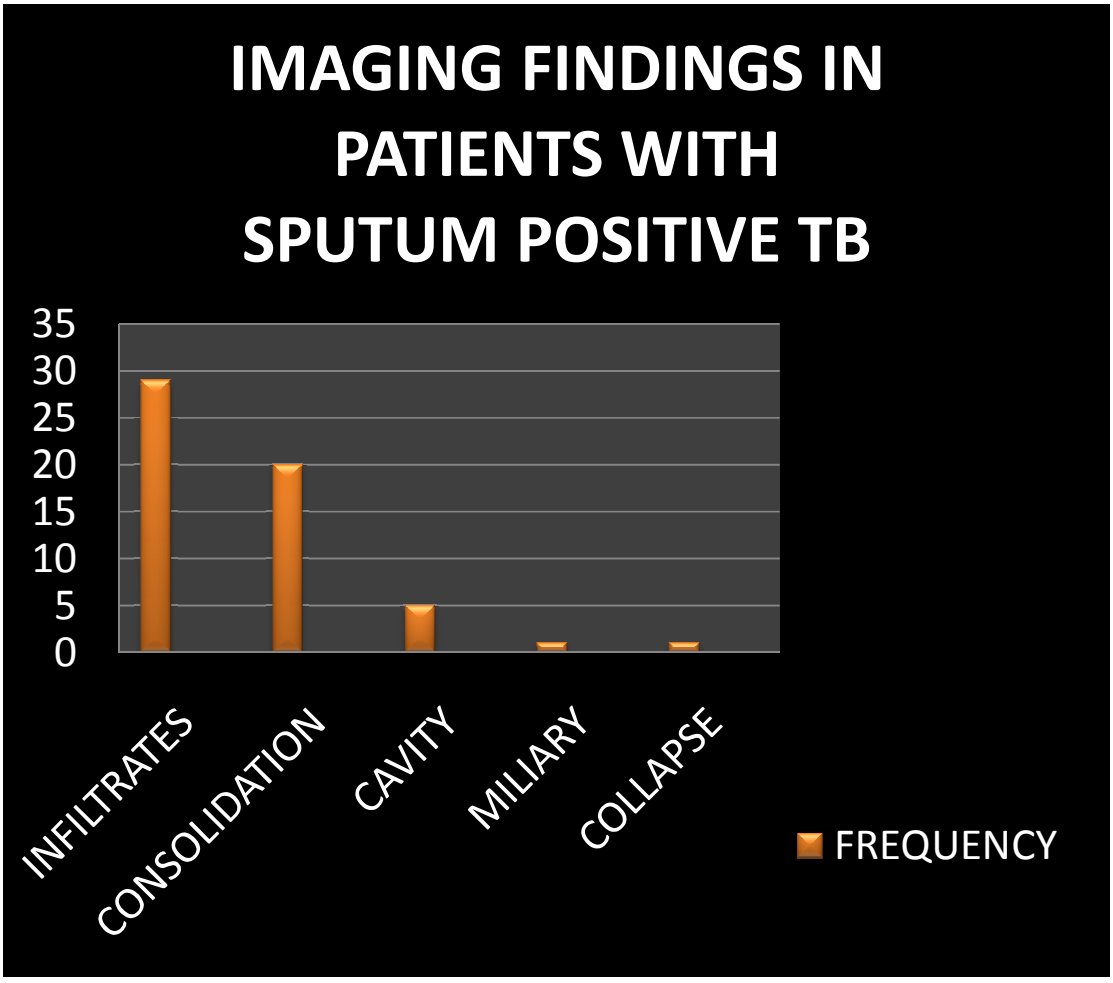
IMAGING FINDINGS IN PATIENTS WITH SPUTUM POSITIVE TB
35
30
25
20
15
10
5
0
INFILTRATES
CONSOLIDATION
CAVITY
MILIARY
COLLAPSE
FREQUENCY

Figure - 6

UNILATERAL VERSUS BILATERAL DISEASE

80.6 % had unilateral pulmonary involvement whereas 19.6 % of the patients had bilateral disease

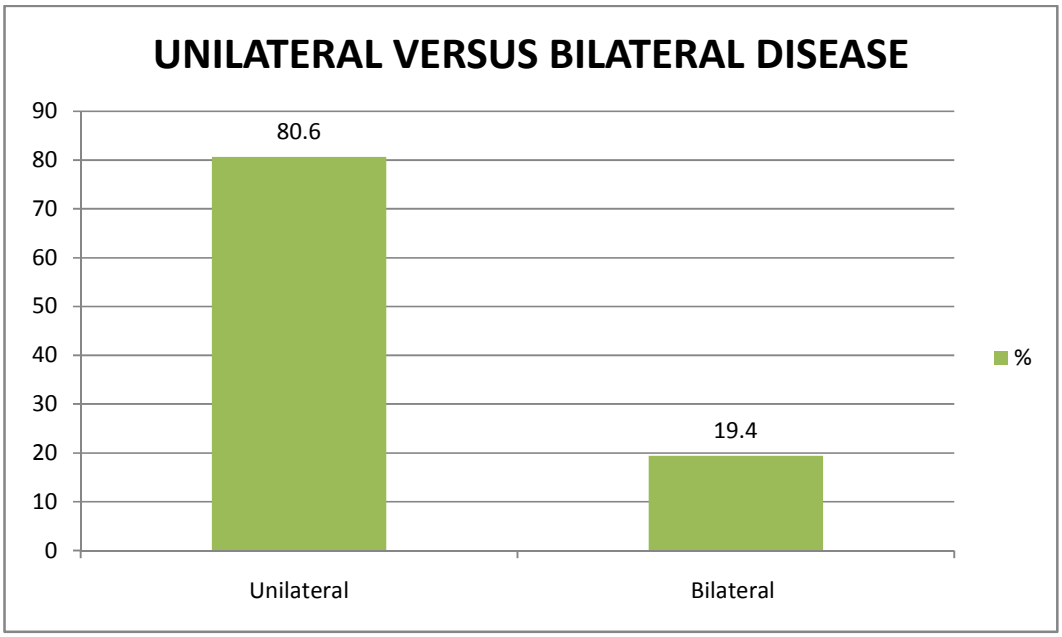

Figure - 7

TABLE : 8

PERCENTAGE OF DIABETICS WITH SPUTUM POSITIVE TB

TOTAL NO. OF CHEST SYMPTOMATICS WHO WERE DIABETICS	20 (19.6 %)
PERCENTAGE OF DIABETICS WITH SPUTUM POSITIVE TB	11 (55 %)

Out of the 102 participants of the study , 20 (19.6 %) had Diabetes Mellitus . Among the twenty diabetics , 11 (55.0 %) had Sputum Positive Tuberculosis .

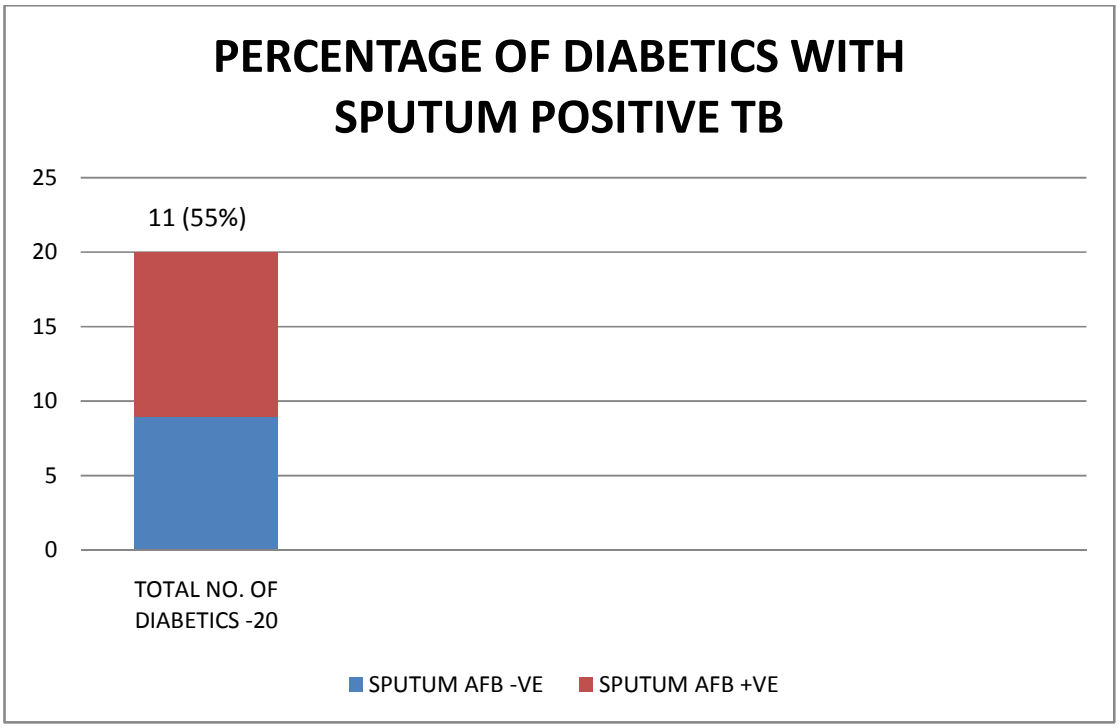
PERCENTAGE OF DIABETICS WITH SPUTUM POSITIVE TB
25
20
15
10
5
0
11 (55%)
TOTAL NO. OF DIABETICS -20
SPUTUM AFB -VE
SPUTUM AFB +VE

Figure - 8

TABLE : 9

PERCENTAGE OF SMOKERS WITH SPUTUM POSITIVE TB

TOTAL NO. OF CHEST SYMPTOMATICS WHO WERE SMOKERS	51 (50%)
PERCENTAGE OF SMOKERS WITH SPUTUM POSITIVE TB	28 (54.9 %)

Out of the 102 participants of the study , 51 (50 %) were smokers. Among them 28 (54.9 %) were sputum positive.

PERCENTAGE OF SMOKERS WITH SPUTUM POSITIVE TB

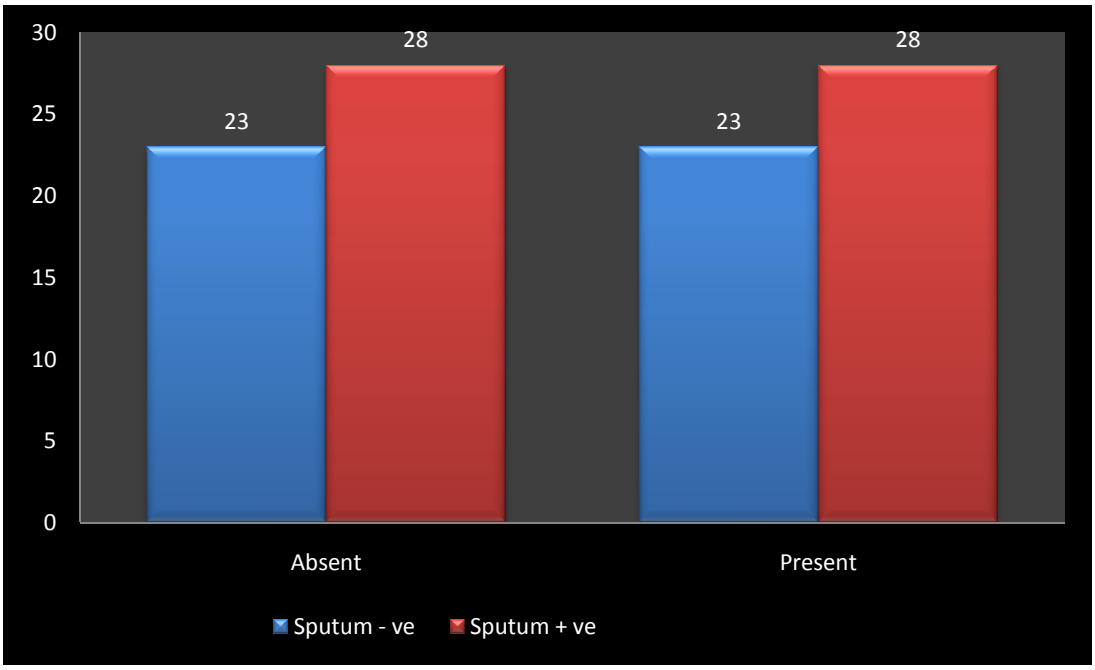

Figure - 9

TABLE : 10

TOTAL NO OF SPUTUM POSITIVE CASES BY ZIEHL - NEELSEN AND FLUORESCENT MICROSCOPY

STAIN	NO OF CASES	%
ZN+VE	39	38.2
FM+VE	56	54.9
FM+VE IN ZN-VE CASES	17	16.67

Ziehl Neelsen detected acid fast bacilli in 39(38.2 %) out of 102 patients whereas Fluorescent Microscopy detected acid fast bacilli in 56 (54.9 %) out of 102 cases .

FM detected 17 more positive cases which were completely missed by Ziehl Neelsen method.

TOTAL NO OF SPUTUM POSITIVE CASES BY ZIEHL-NEELSEN AND FLUORESCENT MICROSCOPY

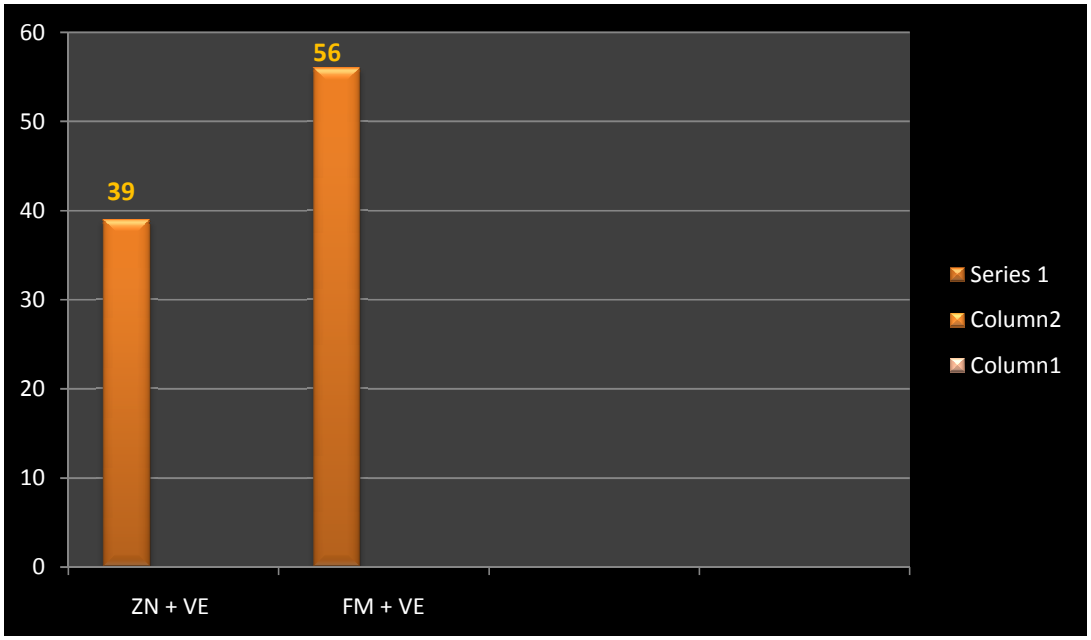

Figure - 10

TABLE 11 :

COMPARISON OF FLUORESCENT STAIN WITH THE GOLD STANDARD ZIEHL NEELSEN STAIN

	ZN POSITIVE	**ZN NEGATIVE**	**TOTAL**
FM POSITIVE	39	17	56
FM NEGATIVE	0	46	46
TOTAL	39	63	100

All the 39 cases which were positive by Ziehl Neelsen method were also positive by Fluorescent Microscopy. In addition, Fluorescent Microscopy also showed positivity in 17 cases which were negative by Ziehl Neelsen method.

TABLE 12 :

EVALUATION OF ZIEHL NEELSEN AND FLUORESCENT MICROSCOPY

	ZN	**FM**
SENSITIVITY(TRUE+VE)	69.6 %	100%
SPECIFICITY(TRUE-VE)	100 %	73%
POSITIVE PREDICTIVE VALUE	100 %	70%
NEGATIVE PREDICTIVE VALUE	73 %	100%

The sensitivity of Fluorescent Microscopy was more (100%) when compared with Ziehl Neelsen method (69.6 %). The number of false negative results (30.3) was also more with Ziehl Neelsen method when compared to Fluorescent Microscopy. Thus in terms of sensitivity Fluorescent Microscopy scores as a better method when compared to Ziehl Neelsen method .

TABLE 13 :

COMPARISON OF ZIEHL NEELSEN AND FLUORESCENT MICROSCOPY

STATISTICAL TESTS	CHI SQUARE	P	2 TAILED P
CHI SQUARE	48.9603		0.0000000001
MC NEMAR CHI SQUARE	15.059		0.001

On comparing two proportions ,

Group 1 ZN = 0.38

Group 2 FM = 0.55

difference = - 0.17 , p value = 0.022

On comparing Ziehl Neelsen method and Fluorescent Microscopy by statistical tests such as Mc nemar ‘s test and Chi square , p value < 0.01 , which is very significant. Thus Fluorescent Microscopy is far superior than Ziehl Neelsen method. Also when comparing two specimens by Ziehl Neelsen vs single home specimen by Fluorescent Microscopy , it is evident that single specimen (early morning sample) when examined by Fluorescent Microscopy has more sensitivity.

DISCUSSION

In India , still tuberculosis continues to be an important disease to be controlled . Almost 20 lakh people are affected in India. Early diagnosis and treatment form the cornerstone of treatment for tuberculosis. Hence there is a need to improve the diagnostic methods. Sputum smear microscopy forms the first line investigation to diagnose pulmonary tuberculosis. This study was conducted to compare the commonly used microscopy method i.e. Ziehl neelsen against fluorescent microscopy as the latter is supposed to have a greater sensitivity.

In this study , 102 chest symptomatic selected according to RNTCP CRITERIA participated . The age and gender distribution of the participants was analysed. In this study , majority of those affected were males (76.8%). The age range of study population was between 15-75 years and the mean age observed was 42.18 years (Table 1). Majority of the patients (50%) were in the middle age group of 31 – 45 years (Table 3).This is in accordance with the data published by A.K.Chakraborty in 2004[27].

The commonest clinical presentation among sputum positive pulmonary tuberculosis patients was found to be cough (82.1%) followed by fever (78.6 %) and loss of weight (66.1 %) . Other usual symptoms observed were breathlessness (44.6 %) , loss of appetite (33.9%) ,

haemoptysis (21.4 %), chest pain(17.9%) and rarely lymphadenopathy (12.5 %)(Table 6).This is in accordance with the survey conducted by Gopi PG [28] in Thiruvallur district ,Tamil Nadu wherein cough more than two weeks(65.6%) was found to be the predominant presenting symptom.

Among the 56 patients whose sputum was positive for tuberculosis , X – ray chest showed infiltrates in 51.8 % of the patients followed closely by tuberculous consolidation in 35.7 % of the patients . Findings like cavity was found in 5 (8.9 %) patients and patterns of collapse (1.7%) and miliary TB (1.7) were found each in only one patient. Sputum for AFB was found to be negative by either method in patients with pleural effusion. 80.6 % had unilateral pulmonary involvement whereas 19.6 % of the patients had bilateral disease .Radiologic manifestations of pulmonary TB are dependent on several host factors, including prior exposure to TB, age, and underlying immune status. In Brazil , 153 patients were studied . the radiological features included parenchymal opacities in 36.6%, cavitation in 36%, pleural effusion in 18%, military pattern in 2.6% [29]

Among the study participants , 20 (19.6 %) had Diabetes Mellitus. Among the twenty diabetics , 11(55.0 %) had Sputum positive Tuberculosis . This is in accordance with the study conducted by Vijay viswanathan et al [30]. The study assessed the incidence and prevalence of diabetes among

patients with tuberculosis. They studied 829 patients out of which 25.3%(207) had diabetes. It was also found that the prevalence was more among males. A higher proportion of patients with co existent tuberculosis and diabetes were smear positive(55.8%). This is due to the fact that prolonged duration of diabetes in a patient impairs both innate and acquired immunity required to fight against the proliferation of mycobacteria.

Out of the 102 participants of the study , 51 (50 %) were smokers. Among them 28 (54.9 %) were sputum positive. In a study conducted by Ariyothai et al in Thailand , it was found that there was increased risk of tuberculosis in smokers. The risk factors included prolonged duration of smoking , smoking more than ten cigarettes per day and initiation of smoking at a very early age [31].

Out of the 102 patients studied , 56 were positive or acid fast bacilli in sputum by either method. Among these positive smears, Ziehl Neelsen detected acid fast bacilli in 39(38.2 %) out of 102 patients whereas Fluorescent Microscopy detected acid fast bacilli in 56 (54.9 %) out of 102 cases . Fluorescent Microscopy detected 17 more positive cases which were completely missed by Ziehl Neelsen method.

Both stains were evaluated and the results were as follows :

ZIEHL NEELSEN METHOD

SENSITIVITY = 69.6%

SPECIFICITY=100%

POSITIVE PREDICTIVE VALUE=100%

NEGATIVE PREDICTIVE VALUE=73%

FLUORESCENT MICROSCOPY

SENSITIVITY = 100%

SPECIFICITY=73%

POSITIVE PREDICTIVE VALUE=70%

NEGATIVE PREDICTIVE VALUE=100%

On comparison of Ziehl Neelsen method and Fluorescent Microscopy by statistical tests such as mc nemar ‘s test and chi square , p value < 0.01 , which is very much significant. Thus Fluorescent Microscopy is far superior than Ziehl Neelsen method. Also when comparing two specimens by Ziehl Neelsen vs single home specimen by Fluorescent Microscopy , it is evident that single specimen (early morning sample) when examined by Fluorescent Microscopy has more sensitivity.

CONCLUSION

This study found that fluorescent microscpy is more sensitive than Ziehl Neelsen method in identifying acid fast bacilli.The fluorescent method was relatively easy to perform and can be done quickly. The mycobacteria could be detected even under lower magnification. Because of this, many fields could be screened in a short time span. The percentage of false negatives was higher when Ziehl Neelsen method was used. Also Fluorescent Microscopy had a greater sensitivity when single home collection specimen was used when compared with Ziehl Neelsen method done in 2 samples as per routine RNTCP. Hence Fluorescent Microscopy fares as a better screening test when compared with Ziehl Neelsen method.

Hence these two approaches, a) Collecting a single early morning sample and b) Screening it using Fluorescent Microscopy could result in rapid as well as accurate diagnosis of Pulmonary Tuberculosis.

The major limiting factor in using a fluorescent microscope is installation and maintenance problems associated with the equipment. The cost of the equipment and also the fluorochrome dyes are also high. To overcome all this problems , LED Fluorescent Microscopes have been introduced . There are several studies demonstrating a higher sensitivity

while using LED Fluorescent Microscopes when compared to the conventional fluorescent and light microscopy.

It is also found that fluorescent microscopy (45%) has a higher sensitivity than the conventional microscopy (29%) in patients co infected with HIV and TB , as fluorescent microscopy identifies more number of pauci bacillary cases , as HIV patients are more likely to be paucibacillary [32].

Fluorescent microscopy is a rapid , useful and a sensitive tool for screening various specimens for mycobacterium tuberculosis. At present it is used only at the level of Intermediate Reference Laboratories. With the advent of LED fluorescent microscopes and demonstration of its superiority over the other techniques in terms of cost effectiveness as well as sensitivity, it should replace the conventional light microscopes in all DOTS centers.

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ABSTRACT

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TOPIC : COMPARISON OF SINGLE SPUTUM SMEAR (HOME COLLECTION) BY FLUORESCENT MICROSCOPY VERSUS TWO SMEARS BY ZIEHL NEELSEN (ROUTINE RNTCP).

PLACE OF STUDY : GOVT. STANLEY MEDICAL COLLEGE AND HOSPITAL, CHENNAI

TYPE OF STUDY : PROSPECTIVE LONGITUDINAL STUDY

DURATION : 6 months

STUDY POPULATION : New chest symptomatics referred to RNTCP cell of Govt.Stanley Medical College for sputum AFB smear microscopy.

AIMS

1) To assess the value of fluorescence microscopy in diagnosing smear positivity among chest symptomatics compared to Ziehl Neelsen microscopy.

2) To assess the value of fluorescent microscopy in picking up additional smear positive cases among smear negative chest symptomatics.

3) Whether the objectives one and two can be achieved with the single home collection sample compared with routine two smear (Ziehl Neelsen) RNTCP smear microscopy.

METHODOLOGY

All patients with chest symptoms will be enrolled in the study after fulfillment of inclusion and exclusion Criteria and getting appropriate consent.

This study is a prospective longitudinal study involving chest symptomatics referred to RNTCP cell of Govt. Stanley medical college for sputum AFB smear microscopy over a period of 6 months. The patients will be advised routine two sputum smear (Ziehl Neelsen) microscopy as per RNTCP guidelines. From the home collection specimen two smears will be prepared, one for Ziehl Neelsen microscopy and the other for fluorescence microscopy. The technicians will not be allowed to cross check the results to avoid bias. Standard stastical methods will be used to assess the correlation between the variables.

BACKGROUND

The global burden of disability and death due to tuberculosis (TB) is immense. The expanding HIV epidemic has further increased the morbidity and mortality due to HIV-TB coinfection. India accounts for one- fifth of the world's new TB cases and two-third of the cases in South East Asia.An estimated 1.9 million cases occur annually and around 0.9 million have sputum positive pulmonary TB.

In developing countries like ours with a large number of tuberculosis (TB) cases and limited resources, the diagnosis of TB relies primarily on smear microscopy for Acid Fast Bacilli (AFB) but its sensitivity is limited in paucibacillarycases. Both smear negative and smear positive pulmonary tuberculous patients are diagnosed in a tertiary care hospital like medical college hospitals with the help of sputum smear microscopy and chest skiagram among chest symptomatics. The conventional Ziehl Neelsen microscopy involves two smears (one home and one spot).

But as the sensitivity of Acid Fast Bacilli smear by Ziehl Neelsen is low, detecting only if 10^5 bacilli are present per ml, there is an urgent need to improve the sensitivity of AFB smear microscopy. Fluorescence microscopy has proved its worthiness in picking up more sputum positivity with less strain to the lab technicians. The area of scanning in fluorescence

microscopy is larger when compared with oil immersion microscope. Conventionally the home collection specimen is considered as superior in picking up positivity when compared to spot specimen. Hence instead of routine two smear microscopy under RNTCP if one home collection specimen can be stained with fluorochrome staining and read under fluorescent microscopy found to be superior in picking up positivity in a high volume set up like medical college hospitals, it will not only minimise the work load to the lab technicians but also will add up more cases diagnosed with accuracy, particularly pauci bacillary cases.

With the revised RNTCP guidelines aiming for higher case detection in the society, this study will help us to probe this hypothesis and if found successful can be implemented in all the medical college hospitals so as to augment the case detection.

Later this can be introduced in the programme at Tuberculosis Unit level to pick up smear positivity among smear negative chest symptomatics in the programme.

INCLUSION CRITERIA :

All chest symptomatics referred for sputum smear Acid Fast Bacilli as per RNTCP criteria

1. Chronic cough >2weeks
2. Evening rise of temperature
3. Unexplained weight loss

EXCLUSION CRITERIA

1. ATT treatment failure patients
2. Relapse of pulmonary tuberculosis
3. ATT defaulters
4. Age < 13 years

PROFORMA

Name :

Age / Sex : Marital Status :

Educational Status : Occupation :

OP No:

Address : Contact No :

SYMPTOMS:- **Duration**

1. Cough
2. Expectoration
3. Haemoptysis
4. Breathlessness
5. Fever
6. Loss of appetite
7. Loss of weight
8. Chest pain

Past History:-

Pulmonary tuberculosis : yes/no ATT intake : duration

Diabetes mellitus: Yes/No duration

Systemic hypertension : Yes/No duration

Personal History:

Diet - vegetarian / non-vegetarian /mixed / fruits / fast food

Appetite - good /poor

Smoking -

Alcohol : - duration - unit

Treatment History :

Contact history :

General Physical Examination:

Pallor

Icterus

Cyanosis

Clubbing

Lymphadenopathy

Pedal oedema

Vitals:

Temperature (in F)

Pulse (/min)

BP (mm of Hg)

Pulse pressure (mm of Hg)

Respiratory Rate

Systemic Examination :

CVS :

RS:

P/A:

CNS:

Investigations : X ray chest PA : U\L (OR) B\L

FINDINGS	YES \ NO	No. of zones involved
Infiltrates		1
Consolidation		2
Cavity		3
Collapse		4
Pleural effusion		5
Calcification		6

Sputum smear for acid fast bacilli

	Spot sample	Home sample
Ziehl neelsen		
Fluorescent microscopy		

OP NO.	age	sex	finding	CXR(UL/BL)- SITE	cough	hem-ptysis	Shortness of breath	fever	loss of appetite	wt loss	chest pain	DM	smoking	HIV contact	lymphnode	ZN spot (+ Y/ -N)	grade	ZN Home (+Y/-N)	grade	PM Home (+Y/-N)	grade	final Y+/N
1306	28	M	miliary tb	b/l	Y	N	N	Y	N	N	N	N	N	N	Y	N	NEG	N	NEG	Y	1+	Y
1308	36	M	infiltrates	b/l - rt uz, lt uz , mz	Y	N	Y	Y	N	N	N	Y	N	N	N	N	NEG	N	NEG	Y	1+	Y
1309	63	M	consolidation	u/l - lt lz	Y	Y	Y	Y	Y	N	Y	Y	N	N	N	Y	SCANTY 2	Y	SCANTY 8	Y	1+	Y
1320	38	F	cavity	u/l - rt uz	Y	N	N	Y	Y	N	N	Y	Y	N	N	Y	SCANTY 9	Y	SCANTY 2	Y	2+	Y
1327	35	M	infiltrates	u/l - lt uz,mz	Y	N	N	Y	N	N	N	Y	Y	N	N	N	NEG	Y	SCANTY 3	Y	1+	Y
1330	19	F	infiltrates	u/l	N	N	N	Y	Y	N	N	N	Y	N	N	Y	2+	Y	2+	Y	2+	Y
1335	23	F	infiltrates	u/l - rt uz	Y	N	N	Y	N	N	N	N	N	N	N	N	NEG	Y	SCANTY 5	Y	1+	Y
1356	34	M	normal		N	N	N	Y	N	Y	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1359	60	M	calcification	u/l - rt uz	Y	N	Y	Y	N	Y	Y	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1062	43	M	infiltrates	b/l - lt mz, rt uz	Y	N	N	N	Y	Y	N	N	Y	N	Y	Y	1+	Y	2+	Y	3+	Y
1355	27	F	cavity	u/l - rt mz	N	N	N	Y	Y	Y	N	N	N	N	Y	Y	SCANTY 3	Y	1+	Y	1+	Y
1357	30	M	calcification	b/l	N	N	N	Y	Y	N	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1154	55	M	normal		Y	Y	N	Y	N	N	Y	Y	N	N	N	N	NEG	N	NEG	N	NEG	N
1254	19	M	cavity	u/l - rt uz	Y	N	Y	Y	Y	N	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1238	26	M	fibrosis	u/l - rt uz	Y	Y	N	N	N	Y	N	N	Y	Y	N	Y	1+	Y	SCANTY 6	N	NEG	N
1367	35	F	consolidation	u/l - lt lz	N	N	Y	Y	Y	Y	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1365	33	F	cavity	u/l - rt mz	Y	N	N	Y	N	N	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1363	46	M	normal		Y	Y	Y	Y	N	N	Y	Y	Y	N	Y	N	NEG	N	NEG	N	NEG	N
1366	40	M	pleural effusion	u/l - ll	Y	N	N	Y	N	N	Y	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1368	52	M	infiltrates	b/l - uz	Y	Y	N	N	N	N	N	Y	N	N	N	N	NEG	N	NEG	N	NEG	N
1416	25	M	infiltrates	u/l - rt	Y	N	N	Y	N	N	N	N	N	Y	N	Y	1+	Y	SCANTY 8	Y	2+	Y
1419	65	M	consolidation	u/l - lt lz	Y	N	N	Y	N	N	Y	Y	N	Y	N	Y	SCANTY 7	Y	1+	Y	1+	Y
1414	42	M	cavity	u/l - lt uz	Y	N	Y	Y	N	N	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1400	49	M	consolidation	u/l - rt uz	Y	Y	Y	Y	N	N	Y	N	N	N	N	N	NEG	N	NEG	Y	1+	Y
1387	46	M	infiltrates	u/l - rt uz	Y	N	N	Y	N	N	N	N	Y	N	Y	Y	2+	Y	2+	Y	2+	Y
1397	38	M	infiltrates	b/l	Y	N	N	Y	N	Y	N	Y	Y	N	N	Y	2+	Y	3+	Y	3+	Y
1430	21	M	consolidation	u/l - lt uz	N	N	N	Y	N	Y	N	Y	Y	N	N	Y	1+	Y	2+	Y	3+	Y
1420	37	M	collapse	u/l - lt lz	Y	Y	Y	Y	Y	Y	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1417	43	M	infiltrates	b/l mz,lz	Y	Y	Y	N	Y	Y	Y	Y	Y	N	N	N	NEG	N	NEG	Y	1+	Y
1540	74	M	consolidation	u/l - lt mz	Y	N	Y	Y	Y	Y	N	N	N	Y	N	Y	1+	Y	2+	Y	1+	Y
1622	53	M	calcification	u/l - rt uz , mz	Y	N	N	Y	N	Y	N	N	Y	Y	N	Y	NEG	N	NEG	N	NEG	N
1627	37	F	consolidation	u/l - rt uz	Y	N	Y	Y	N	Y	N	N	Y	N	Y	Y	1+	Y	1+	Y	3+	Y
1628	42	M	consolidation	u/l - lt uz	N	N	Y	N	Y	Y	N	N	Y	N	N	Y	1+	Y	2+	Y	3+	Y
1660	40	M	infiltrates	b/l - rt uz, lt uz,mz	Y	N	N	Y	N	Y	N	N	Y	N	N	Y	2+	Y	1+	Y	1+	Y
1572	37	F	consolidation	u/l - lt lz	N	N	Y	N	Y	Y	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1617	68	M	collapse	u/l - rt lz	Y	N	Y	Y	N	Y	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1590	70	M	fibrosis	b/l - uz	Y	Y	Y	Y	Y	Y	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1674	35	M	calcification	b/l - rt uz, lt mz,lz	N	N	N	N	N	Y	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1683	48	M	consolidation	u/l - lt uz	Y	N	N	N	Y	N	N	N	Y	N	Y	Y	2+	Y	1+	Y	3+	Y
1684	42	M	infiltrates	u/l - rt uz	Y	N	Y	Y	N	Y	Y	N	Y	N	N	Y	1+	Y*	3+	Y	3+	Y
1688	24	M	pleural effusion	u/l - rt	Y	N	Y	Y	Y	Y	N	N	N	N	N	Y	1+	N	NEG	N	NEG	N
1696	21	F	infiltrates	u/l - rt	N	N	Y	N	Y	Y	N	N	N	N	N	Y	3+	Y	1+	Y	3+	Y
1467	42	M	consolidation	u/l - rt lz	Y	Y	Y	Y	Y	N	Y	N	Y	Y	N	Y	2+	Y	1+	Y	2+	Y
1454	30	F	infiltrates	u/l - lt	N	N	Y	Y	N	N	Y	N	N	N	N	Y	1+	Y	1+	Y	1+	Y
1463	55	M	consolidation	u/l - rt uz	Y	N	N	N	Y	Y	Y	N	Y	N	N	Y	2+	Y	1+	Y	1+	Y
1477	37	M	cavity	u/l - rt mz	Y	N	Y	N	Y	Y	N	N	N	N	N	Y	2+	Y	1+	Y	2+	Y
1424	43	M	consolidation	u/l - lt uz	Y	N	Y	Y	Y	Y	N	N	Y	N	N	Y	2+	Y	3+	Y	1+	Y
1431	60	M	collapse	u/l - rt uz	Y	Y	Y	Y	N	Y	N	Y	Y	N	N	Y	1+	Y	2+	Y	2+	Y
1437	45	M	consolidation	u/l - lt lz	Y	N	Y	Y	Y	Y	N	N	N	N	N	Y	2+	Y	1+	Y	1+	Y
1479	48	M	cavity	u/l - rt uz	Y	N	N	Y	N	N	Y	N	Y	N	N	Y	1+	Y	2+	Y	2+	Y
1450	43	M	consolidation	u/l - rt uz	Y	N	Y	Y	Y	N	Y	N	Y	N	N	Y	1+	Y	2+	Y	2+	Y
1517	20	M	pleural effusion	lt	N	N	N	Y	N	Y	N	N	N	N	N	N	NEG	N	NEG	N	NEG_5	N

1498	45	M	infiltrates	u/l - rt uz	Y	N	Y	N	Y	Y	N	N	Y	N	N	N	NEG	N	NEG	Y	1+	Y
1500	61	M	calcification	h/l	Y	N	Y	Y	Y	N	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1513	55	M	normal		Y	N	N	Y	N	N	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1533	57	M	infiltrates	h/l - rt uz, lt mz,lz	Y	Y	Y	Y	Y	N	N	N	Y	N	N	Y	3+	Y	1+	Y	3+	Y
1564	50	M	consolidation	u/l - lt mz	Y	N	N	Y	N	Y	N	N	N	N	N	Y	3+	Y	1+	Y	2+	Y
1605	63	M	infiltrates	u/l - rt lz	Y	N	Y	Y	N	Y	N	N	Y	N	N	Y	3+	Y	2+	Y	1+	Y
1546	55	M	infiltrates	h/l - rt uz,mz, lt uz	Y	N	N	N	Y	Y	N	Y	Y	Y	Y	N	NEG	N	NEG	Y	3+	Y
1598	48	M	consolidation	u/l - lt lz	Y	Y	Y	Y	Y	Y	N	N	N	N	Y	Y	1+	Y	2+	Y	2+	Y
1575	30	F	infiltrates	u/l - rt lz	Y	N	N	Y	Y	Y	Y	N	N	N	N	Y	1+	Y	2+	Y	1+	Y
1579	35	M	infiltrates	u/l - rt uz	Y	N	N	Y	N	N	N	N	N	Y	Y	Y	2+	Y	1+	Y	2+	Y
1473	35	F	pleural effusion	u/l - rt	Y	N	N	Y	Y	N	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1487	61	M	calcification	h/l		N	Y	N	Y	Y	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1573	17	M	normal		Y	N	N	Y	N	N	Y	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1539	56	M	normal		Y	N	N	N	Y	Y	Y	Y	Y	N	N	N	NEG	N	NEG	N	NEG	N
1748	58	M	consolidation	u/l - lt lz	Y	N	N	Y	N	Y	N	Y	Y	N	N	N	NEG	N	NEG	N	NEG	N
1704	70	M	collapse	u/l - rt uz	Y	Y	Y	N	Y	N	Y	Y	Y	N	N	N	NEG	N	NEG	N	NEG	N
1739	24	M	pleural effusion	u/l - rt	Y	N	Y	N	N	Y	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1762	36	M	normal		Y	N	N	Y	N	N	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1902	47	M	consolidation	u/l - lt uz	Y	Y	N	Y	Y	Y	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1883	54	M	normal		Y	N	N	Y	Y	N	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1905	25	F	cavity	u/l - rt uz	Y	N	N	Y	N	N	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1781	61	M	infiltrates	h/l - rt mz , lt	Y	Y	Y	Y	N	Y	Y	Y	Y	N	N	N	NEG	N	NEG	N	2+	Y
1775	42	M	fibrosis	u/l - rt uz	Y	N	N	N	N	N	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1723	39	M	calcification	u/l - rt uz	Y	N	N	Y	N	Y	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1764	33	F	pleural effusion	u/l - lt	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1759	65	M	calcification	h/l	Y	N	Y	N	N	N	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1708	45	M	infiltrates	u/l - rt lz	Y	N	N	Y	N	N	Y	Y	Y	N	N	N	NEG	N	NEG	Y	3+	Y
1817	41	M	consolidation	u/l - lt uz	Y	N	N	Y	N	N	N	Y	Y	N	N	N	NEG	N	NEG	Y	2+	Y
1793	32	M	infiltrates	u/l - rt lz	Y	N	N	Y	N	N	N	Y	Y	N	N	Y	1+	N	NEG	Y	2+	Y
1827	37	M	consolidation	u/l - rt uz	Y	N	N	Y	N	N	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1882	60	M	consolidation	u/l - rt lz	Y	N	N	Y	N	N	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1934	51	M	normal		Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	NEG	N	NEG	N	NEG	N
1931	40	M	fibrosis	u/l - rt uz	Y	N	Y	Y	Y	N	N	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1814	49	F	consolidation	u/l - rt mz	Y	N	N	N	N	Y	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1755	41	M	cavity	h/l - uz	Y	N	Y	Y	Y	Y	N	N	N	N	Y	Y	2+	N	NEG	Y	3+	Y
1838	31	F	normal		N	N	Y	N	N	N	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1956	29	F	consolidation	u/l - rt uz	N	N	Y	N	Y	Y	N	N	N	N	N	N	NEG	N	NEG	Y	1+	Y
1935	66	M	cavity	h/l - rt uz, lt uz	Y	N	Y	Y	N	Y	Y	N	Y	N	N	N	NEG	N	NEG	N	NEG	N
1837	21	M	normal		N	N	Y	N	Y	N	N	N	N	N	N	N	NEG	N	NEG	N	NEG	N
1793	16	M	infiltrates	u/l - rt	N	N	Y	Y	N	N	N	N	N	N	N	N	NEG	Y	3+	Y	3+	Y
4575	27	F	consolidation	u/l - h mz	N	N	Y	Y	N	Y	N	N	N	N	N	Y	SCANTY 8	Y	1+	Y	1+	Y
4616	53	M	consolidation	h/l - rt uz, lt lz	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N	N	1+	Y	2+	Y	1+	Y
4535	45	M	infiltrates	u/l - rt uz	Y	N	Y	N	Y	N	N	N	Y	N	N	N	2+	Y	1+	Y	1+	Y
4536	28	F	infiltrates	u/l - lt uz,mz	Y	N	N	Y	N	Y	N	N	N	N	Y	N	1+	Y	2+	Y	2+	Y
4528	43	M	consolidation	u/l - rt uz	Y	Y	Y	N	Y	N	N	Y	N	N	N	Y	3+	Y	1+	Y	1+	Y
4509	70	F	infiltrates	u/l - rt lz	N	N	Y	Y	N	N	Y	N	N	y	N	N	NEG	N	NEG	Y	2+	Y
4404	26	M	infiltrates	u/l - rt uz	N	N	Y	N	Y	Y	N	N	N	y	N	Y	1+	Y	SCANTY 3	Y	2+	Y
4502	36	M	consolidation	u/l - lt uz	Y	N	Y	N	Y	Y	Y	N	N	N	Y	N	SCANTY 5	Y	1+	Y	1+	Y
4577	30	M	infiltrates	u/l - h mz	Y	N	N	Y	Y	Y	N	N	N	N	Y	Y	2+	Y	3+	Y	3+	Y
1384	32	F	infiltrates	u/l - lt	N	N	Y	Y	Y	Y	N	N	N	Y	N	N	NEG	N	NEG	Y	1+	Y

CONSENT FORM

1. I agree to participate in the study entitled "COMPARISON OF SINGLE SPUTUM SMEAR (HOME COLLECTION) BY FLUORESCENT MICROSCOPY VERSUS TWO SMEARS BY ZIEHL NEELSEN (ROUTINE RNTCP) IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS".
2. I confirm that I have been told about this study in my mother tongue and have had the opportunity to ask questions
3. I understand that my participation is voluntary and I may refuse to participate at any time without giving any reasons and without affecting my benefits.
4. I agree not to restrict the use of any data or results that arise from this study.

Name of the participant :

Sign / Thumb print :

Sign of the Investigator

ABBREVIATIONS

AFB	**-**	Acid Fast Bacilli
AO	**-**	Auramine O
FM	**-**	Fluorescent microscopy
TB	**-**	Tuberculosis
ZN	**-**	Ziehl Neelsen
WHO	-	World Health Organization
RNTCP	-	Revised National Tuberculosis Control Programme
HIV	-	Human Immuno Deficiency Virus
DOTS	-	Directly Observed Treatment Short course

INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : Comparison of single sputum smear (Home Collection) By fluorescent microscopy versus two smears by ziehl neelsen (Routine RNCTP)

Principal Investigator : Dr. S. Geethalakshmi

Designation : PG in MD (GM)

Department : Department of Gen. Med.
Government Stanley Medical College,
Chennai-1

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 06.03.2012 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

MEMBER SECRETARY,
IEC, SMC, CHENNAI